Optimization of pickled herring production - Approaches for process and quality control

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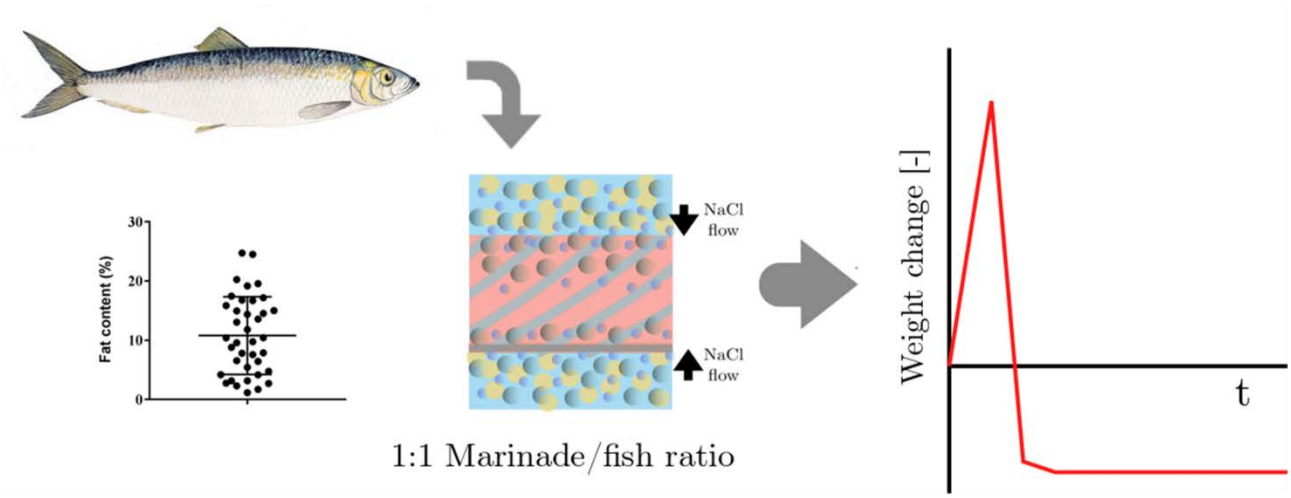

Maria Helbo Laub-Ekgreen
PhD thesis
May 2018

DTU Food
National Food Institute

OPTIMIZATION OF PICKLED HERRING PRODUCTION

Approaches for process and quality control

PhD Thesis

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Data sheet

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Preface and acknowledgements

This thesis entitled "*Optimization of pickled herring production - approaches for process and quality control*" was carried out at the National Food Institute, Technical University of Denmark. The work was started in May 2014 and continued until May 2018, interrupted for one year of maternity leave. Associate professor Stina Frosch mainly supervised the project until May 2016 followed by senior professor Flemming Jessen. The co-supervisors of the project were Bo Munk Jørgensen (Associate Professor) and Brais Martinez Lopez (Assistant professor) from May 2016. This work was supported by *Green Development and Demonstration Program, GUDP, Ministry of Food, Agriculture and Fisheries, Denmark*

First, I would like to thank my supervisors: Stina, for giving me this opportunity and believing in me. Flemming for taking on this project and for fruitful scientific inspiration and talks and for always being positive and optimistic. A thank you goes to Brais for sharing his passion for modelling and good talks during this project. A special thank is given to Thomas Skov from Copenhagen University (CAT group) for valuable discussions, encouragement and keeping high spirit. Last, but not least at thank to Bo for valuable discussion of multivariate data analysis. A thank to Skagerak Pelagic A/S for supplying raw materials and good discussions.

All my colleagues at FPE are thanked for their help and scientific inspirations as well as for providing a good working environment with great coffee runs to Ricco's and fun social gatherings.

Above all, I will express my deepest thankfulness to my family for their encouragement particular during the last intensive months of writing. This especially goes out to my husband, Lasse. Thanks to my children, Alfred and Liva, for adding extra meaning to life and for making me work even more efficient.

This project has been a journey for me and I have learned the strengths and weaknesses of myself.

May 17, 2018

Maria Helbo Laub-Ekgreen

Abstract

Manufacturing of high-quality pickled herring products requires a thorough understanding of the raw material as well as the process. Commonly, the industrial process is based on traditional principles and practical experience. In order to optimize the industrial process, it is essential to understand how the process parameters relate to the raw material characteristics (such as seasonal variation) and affects the product quality and yield. Product yield is one of the main aspects of a profitable production of pickled herring products, and attaining a high product yield without compromising food safety and quality is desired.

The aim of this thesis is to contribute with knowledge to optimize the industrial production of pickled herring products. The experimental work can be divided into three main subjects: i) obtaining a deeper knowledge about the influence of different parameters (biological/process) on the product yield, ii) studying the salt and water diffusion in herring muscle during dry salting and brining to be used in a model describing these phenomena and iii) investigating the use of Near-infrared (NIR) spectroscopy to determine the salt content in herring products.

Regarding the first subject mentioned above, several storage experiments were conducted using single herring fillets, where brining time, marinade composition and marinating time varied. Conducting experiments on single fillets revealed a correlation between fat content and weight change during processing. The greatest weight change occurred within the first 2-3 days and only minor changes were seen during storage up to one year (Paper I).

Different salting conditions were applied to herring fillets in order to investigate the salt and moisture transfer in herring during processing by experiments of global and local concentration profiles. The average salt and moisture changes in fillets during brining were studied under conditions similar to industrial processes using a brine-to-fish ratio of 1:1. An average diffusion coefficient for salt was found, being 2.31×10^{-9} m^2s^{-1} (Paper I). Local concentration profiles were obtained to study the counter diffusion of salt and moisture under the different salting conditions and to determine the diffusion coefficients of salt and water that were used to develop the model. The model can be used for prediction of salt and moisture distribution and equilibrium times (Paper II).

NIR spectroscopy was investigated as a potential method for process control in the herring marinating process. A principal component analysis (PCA) of the spectral data of herring marinade showed that the first principal component was related to the change in salt

concentration during processing, and it was possible to calibrate models for prediction of the salt concentration in the marinade and in the herring fillets under the prerequisite that the system was in equilibrium. We concluded that NIR is a good alternative for the time consuming and destructive methods for salt determination often used in the industry (Paper III).

In conclusion, this work has added knowledge about the variation in product yield, developed models that can be used to predict salt concentration and immersion time and we have proposed the use of NIR to achieve fast and non-destructive salt determinations which all-together aid in optimizing the process in the industry.

Resumé (Danish)

Produktion af marinerede sild med høj kvalitet kræver en grundig forståelse af råmaterialet samt produktionsprocessen. Produktionsprocessen i industrien er ofte baseret på traditionelle principper og praktisk erfaring. For at kunne optimere processen er det essentielt at forstå, hvordan de forskellige procesparametre relaterer sig til de forskellige biologiske faktorer i råmaterialet (sæsonvariation mm.), og hvordan dette påvirker slutproduktets kvalitet samt udbyttet.

Formålet med denne afhandling er at skabe ny viden på baggrund af videnskabelige forsøg og modeller, der kan optimere produktion af marinerede sild i industrien. Det eksperimentelle arbejde kan deles op i tre hovedemner: 1) at opnå en bedre forståelse af hvordan de forskellige proces- og biologiske parametre påvirker produktionsudbyttet, 2) at studere salt- og vanddiffusionen i sildemuskler under tørsaltning og i saltlage for at kunne anvende dette i en model, der bekriver denne biologiske proces, 3) at undersøge om nærinfrarød (NIR) spektroskopi kan anvendes til at bestemme saltindholdet i sildeprodukter.

Vedr. det første emne nævnt ovenfor, udførte vi en række lagringseksperimenter ved brug af individuelle sildefileter, hvor tiden i saltningslagen, tiden i marinaden og marinadens sammensætning blev varieret. Forsøgene på individuelle sildefileter viste en korrelation mellem fedtindholdet og vægtændringen under lagring. Den største vægtændring skete i de første 2-3 dage, og der skete kun få yderligere vægtændringer ved op til at års lagring (Artikel I).

Globale og lokale koncentrationsprofiler i sildefileter blev opsamlet ved forskellige saltningsmetoder for at kunne undersøge salt- og vandiffusionen under saltning. Det gennemsnitlige indhold af salt og vand i fileterne blev undersøgt under forhold, der ligner industriens ved brug af en lage/fisk ratio på 1:1. Diffusionskoefficienten for salt var i gennemsnit $2{,}31 \times 10^{-9}$ m^2s^{-1} (Artikel I). Lokale koncentrationsprofiler blev anvendt til at studere den koblede diffusion af vand og salt under de forskellige saltningsforhold samt til at bestemme diffusionskoefficienterne for salt og vand, som blev brugt til at udvikle modellen. Modellen kan anvendes til at beskrive fordelingen af vand og salt i silden samt tid til ligevægt (Artikel II).

Vi undersøgte NIR spektroskopi som en mulig metode til proceskontrol i sildemarineringsprocessen. Principal komponent analyse (PCA) af de spektrale data fra sildemarinaden viste, at den første PC var relateret til ændringen i saltkoncentrationen under marinering. Det var muligt at kalibrere modeller til at beskrive saltkoncentrationen i marinaden og i sildefileterne under forudsætning af, at systemet var i ligevægt. Vi vurderer derfor, at NIR

spektroskopi er et godt alternativ til de mere tidskrævende og destruktive metoder, der oftest bruges i industrien til saltbestemmelser (Artikel III).

Baseret på arbejder i denne afhandling, kan vi konkludere at vi: 1) har øget vores viden om variationen i produktudbyttet ved produktion af marinerede sild, 2) har udviklet modeller som kan bruges til beskrive saltkoncentrationen og marineringstiden og 3) har skabt grundlag for brugen af NIR som hurtig og ikke-destruktiv metode til at bestemme saltkoncentrationer. Samlet vil resultaterne af dette projekt kunne anvendes til at optimere produktionen af marinerede sild i industrien.

Table of contents

List of abbreviations

GHP	Good hygiene practices
GMP	Good Manufacturing Practices
LV	Latent Variables
MRI	Magnetic Resonance Imaging
MSC	Multiple Scatter Correction
NaCl	Sodium Chloride
NIR	Near-infrared Spectroscopy
NIRS	NIR Spectroscopy
NMR	Nuclear Magnetic Resonance
PC	Principal Component
PCA	Principal Component Analysis
PLS	Partial Least Squares
RMSE	Root Mean Square Error
RMSEC	Root Mean Square Error of Calibration
RMSECV	Root Mean Square Error of Cross Validation
RMSEP	Root Mean Square Error of Prediction
SNV	Standard Normal Variate
SPC	Statistical Process Control
TPA	Texture Profile Analysis
WPS	Water phase salt (g/100 g)

List of manuscripts

This thesis is based on research reported in the following papers, which in the text are referred to as Paper I-III.

Paper I

Maria Helbo Laub-Ekgreen, Brais Martinez-Lopez, Stina Frosch & Flemming Jessen (2018).The influence of processing conditions on the weight change of single herring (*Clupea harengus*) fillets during marinating. *Food Research International*, 108 p. 331-338.

Paper II

Maria Helbo Laub-Ekgreen, Flemming Jessen & Brais Martinez-Lopez (2018). Mechanistic modelling of the coupled salt and water transport in herring during brining and curing. Manuscript submitted to *Journal of Food Engineering* (November 2018).

Paper III

Maria Helbo Laub-Ekgreen, Brais Martinez-Lopez, Flemming Jessen & Thomas Skov (2018). Non-destructive measurement of salt using NIR spectroscopy in the herring marinating process, *LWT- Food Science and Technology*, 97 p. 610-616.

Chapter 1: **Introduction**

This chapter introduces the motivation, objectives and outline of the PhD thesis.

1.1 Motivation for the study

The Atlantic herring (*Clupea harengus*) is very important for the Danish fishing industry. Denmark is one of EU´s largest players in the herring industry and the Danish industry in dominated by a few large herring manufacturing companies. The herrings are caught in the nearby seas around Denmark and mainly manufactured into marinated, also known as pickled herring, and salted products, which are sold as semi-manufactured products to different factories in EU and mainly consumed in the Northern European countries (Karl, Roepstorff, Huss, & Bloemsma, 1995).

The manufacturing of high-quality pickled herring products requires a thorough understanding of the raw material as well as the process. However, the marinating procedures are commonly based on traditional principles and practical experience (Welti-Chanes, Vergara-Balderas, & Bermúdez-Aguirre, 2005). Product yield is one of the main aspects of a profitable production of marinated herring products, and attaining a high product yield without compromising food safety and quality is desired. A product loss of 5-30 % after the marinating process is commonly observed resulting in a total loss of 5-6000 tonnes of product every year (Personal communication, Skagerak Pelagic). The reasons for the change in yield are often not analysed or documented in the industry.

During processing of herring into marinated products a change in weight occurs because of the effect of salt and acetic acid (Rodger, Hastings, Cryne, & Bailey, 1984). The magnitude of the weight change depends on the concentration of the solutes, the processing conditions and lastly the quality of the raw material. It is therefore of major interest to the industry to know more about the effect of these parameters.

A feasible approach to investigate these parameters could therefore include experiments on individual herrings in order to relate the raw material composition to the process related changes. Furthermore, developing models that accurately describe the herring marinating process relating to different raw material characteristics and manufacturing practices would be of great value for the industry. Ideally, the model should be able to predict the appropriate

salting and marinating conditions for obtaining safe products in the shortest possible time. In order to develop such models investigations of the diffusion of salt/acetic acid in the herring muscle should be conducted.

The salt content of marinated herring products is one of the most important quality and safety parameters, and salt content is determined at different steps during the production. Standard methods are often destructive and labour intensive, where products are taken out of the production. Moreover, it is often impossible to automate. Spectroscopic methods such as NIR spectroscopy has previously been used to determine salt content in foodstuff (Galvis-Sanchez, Tóth, Portela, Delgadillo, & Rangel, 2011; Huang, 2001; Lin, Cavinato, Huang, & Rasco, 2003) and could prove as a promising method for fast process control in the marinated herring production.

1.2 Objectives of the study

The overall aim of this PhD project is to optimize the herring marinating process resulting in a more controlled process, a better utilization of the raw material and sustaining the product safety and quality. In order to fulfil the project aim different approaches are applied:

- Obtaining a deeper knowledge about the influence of different parameters (biological/process) on the product yield.
- Studying the salt and water diffusion in herring muscle during dry salting and brining to be used in a model describing these phenomena.
- Investigating the use of NIR spectroscopy in process control.

The work was conducted in close collaboration with the herring manufacturing industry (Skagerak Pelagic A/S, Skagen, Denmark). Figure 1.1 shows the parts of the herring marinating process we have focused on in our studies. In the brining process, we studied the salt and moisture transfer as well as the use of NIR spectroscopy to monitor the salting process in order to achieve a better control of the process. For the marinating process, we studied the effect of different process parameters as well as the natural variation of the raw material on the product yield in order to gain a deeper knowledge about the process.

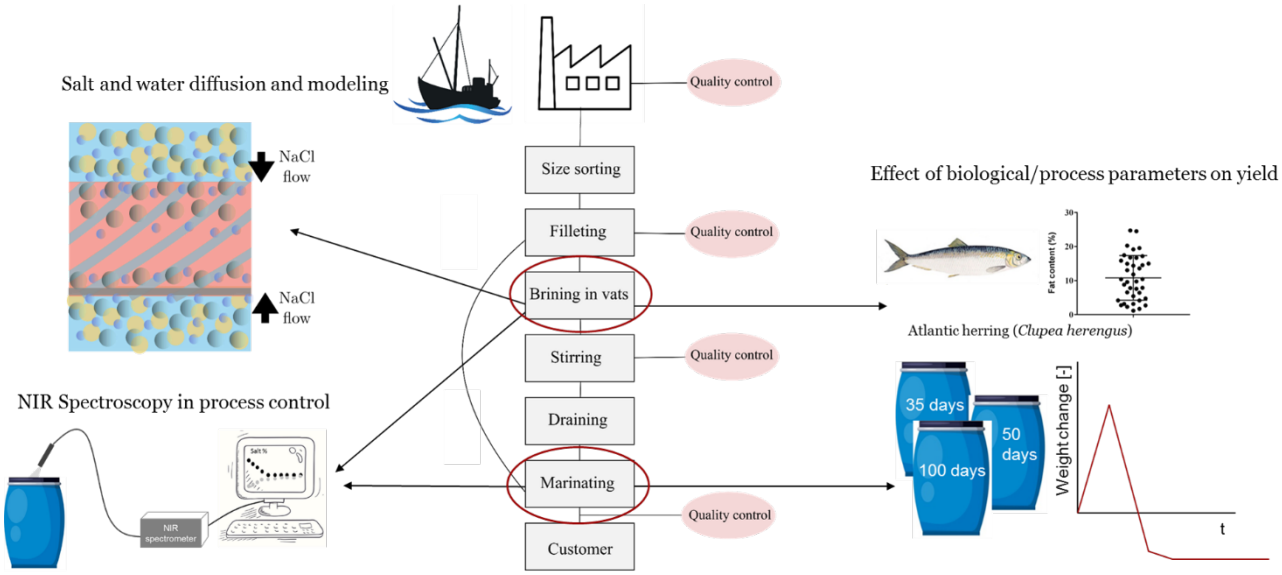

Figure 1.1: Overview on the conducted research with a viewpoint from the herring marinating process.

1.3 Outline

The thesis is structured in six chapters describing the background of the work, the experimental studies and a short discussion of use of the obtained results. Some of the material in this thesis has been published or submitted for publication and the main results are presented in chapter 4 along with results that were not included in the papers. Having introduced the motivation and objectives of the PhD thesis in Chapter 1, Chapter 2 provides the theoretical background for the work and Chapter 3 provides an overview of the experimental work that has been carried out during this project, which forms the basis of the analyses described in Chapter 4. Chapter 4 describes the main results obtained in this project and is divided into two sections. The first section presents the studies of the salting/brining process of herring fillets focusing on obtaining global and local concentration profiles of the salt and water flux in herring fillets during brining. The second section presents the studies of the combined brining and marinating process focusing on the fillet weight change during marinating and the effect of an intermediate brining step. Moreover, the use of NIR for process control is evaluated. In chapter 5, the conclusion is presented. Finally, the future perspectives are presented in chapter 6.

The three papers are found in Appendix 1. Paper I describes the influence of processing conditions on the weight change of single herring (*Clupea harengus*) fillets during marinating. Paper II describes the distribution of salt and water concentration in the herring fillets during brining and dry salting and modelling of the diffusion of salt and water. Paper III investigated the use of NIR spectroscopy as a non-destructive method to determine the salt content in marinade/fish in the herring marinating process.

Chapter 2: **Background**

This chapter introduces background of the study with focus on the herring marinating process, the raw material, the effect of salt and acetic acid, the mass transfer mechanisms as well as an introduction to Near-infrared spectroscopy.

2.1 Raw material

The raw material used in this project was Atlantic herring (*Clupea harengus*) caught in the seas around Denmark. The herring is a pelagic fish species, which vary in length from approximately 20 to 40 cm. It is found from 2 to 400 m below the sea surface and when maturing it moves towards coastal waters in larges schools to spawning grounds (Stroud, 1972). The variation in the fat content is known to be the primary quality parameter for different herring products, and the fat content is found to vary between 1.3 % to 25.7 % between catches and is correspondingly high within catches (Nielsen, Hyldig, Nielsen, & Nielsen, 2005a). Variation in the fat content comes from seasonal effect on the feed availability and water temperature and follows the cycle of maturation (Aidos, van der Padt, Luten, & Boom, 2002; Nielsen et al., 2005a; Szlinder-Richert, Usydus, Wyszyński, & Adamczyk, 2010; Timberg, Koppel, Kuldjärv, & Paalme, 2011). The variation in fat within a batch can be due to mixing of stocks and the presence of an uneven age distribution of the herring (Lane, Westgate, & Koopman, 2011; Nielsen et al., 2005a; Rajasilta, 1992). Herrings have starvation periods when they are spawning or migrating, and the fat content in the herrings will have a minimum at the time of spawning, which is illustrated in Figure 2.1 for spring spawning herrings.

The change in fat content is also reflected in the change in water content as the sum of these two counts for approximately 80 % of the herring fillet (Nielsen et al., 2005a). Water is the main constituent of the fish muscle and is mainly found in the myofibrils, that accounts for app. 60-80 % of the total protein in fish (Delbarre-Ladrat, Chéret, Taylor, & Verrez-Bagnis, 2006). Most of the water is associated to the proteins, at different degrees, and only a small fraction of the water is tightly bound. The interaction of water and protein affects the functional properties such as the water holding capacity and texture (Chou & Morr, 1979; Dunajski, 1979; Nguyen, Thorarinsdottir, Gudmundsdottir, Thorkelsson, & Arason, 2011). The herring is of great

importance for the Danish fishing industry and mainly processed into salted, marinated or smoked products (Birkeland, Sivertsvik, Nielsen, & Skåra, 2005; Stroud, 1972).

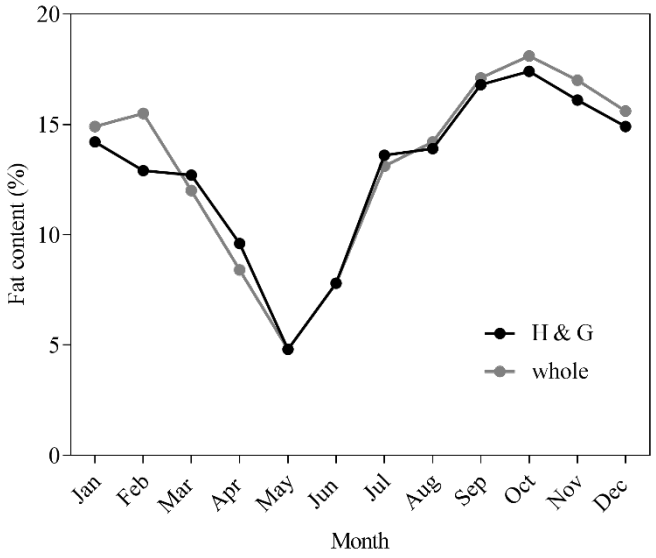

Figure 2.1: Fat content in whole and headed and gutted (H & G) herring caught at different times during the years 1979-1987 adopted from Einarsson (1988).

2.2 Processing

Various herring preservation techniques exist, including traditional salting and marinating using salt and an organic acid. Differences in salt, acid concentration and processing (i.e. storage temperature and storage time) have resulted in many versions of preserved herring. Herring fillets or pieces marinated in a solution of acetic acid and sodium chloride are considered semi-preserved products (Meyer, 1965), which are defined as fish product with a salt concentration >6 % in the water phase or a pH value <5.0 and storage a temperature ≤10 °C (Huss, Ababouch, Gram, Huss, Ababouch, & Gram, 2004). The salting and marinating procedures often varies and depends on local tradition of the manufacturing country and company. However, a common denominator is to reduce the pH value to slow the action of bacteria and enzymes and make the fish available for consumption most of the year (McLay, 1971; Rodger et al., 1984; Szymczak, 2011). In a typical German process, the herring fillets are marinated directly in a strong solution of salt and acetic acid. In Denmark the use of an intermediate brining step is common practice (Karl et al., 1995), which is believed to improve quality and weight yield of the herring fillets (Birkeland et al., 2005). An intermediate brining step is also found in the process of heavy salted cod (Thorarinsdottir, Arason, Sigurgisladottir, Gunnlaugsson, et al., 2011) and smoked salmon (Gallart-Jornet et al., 2007b).

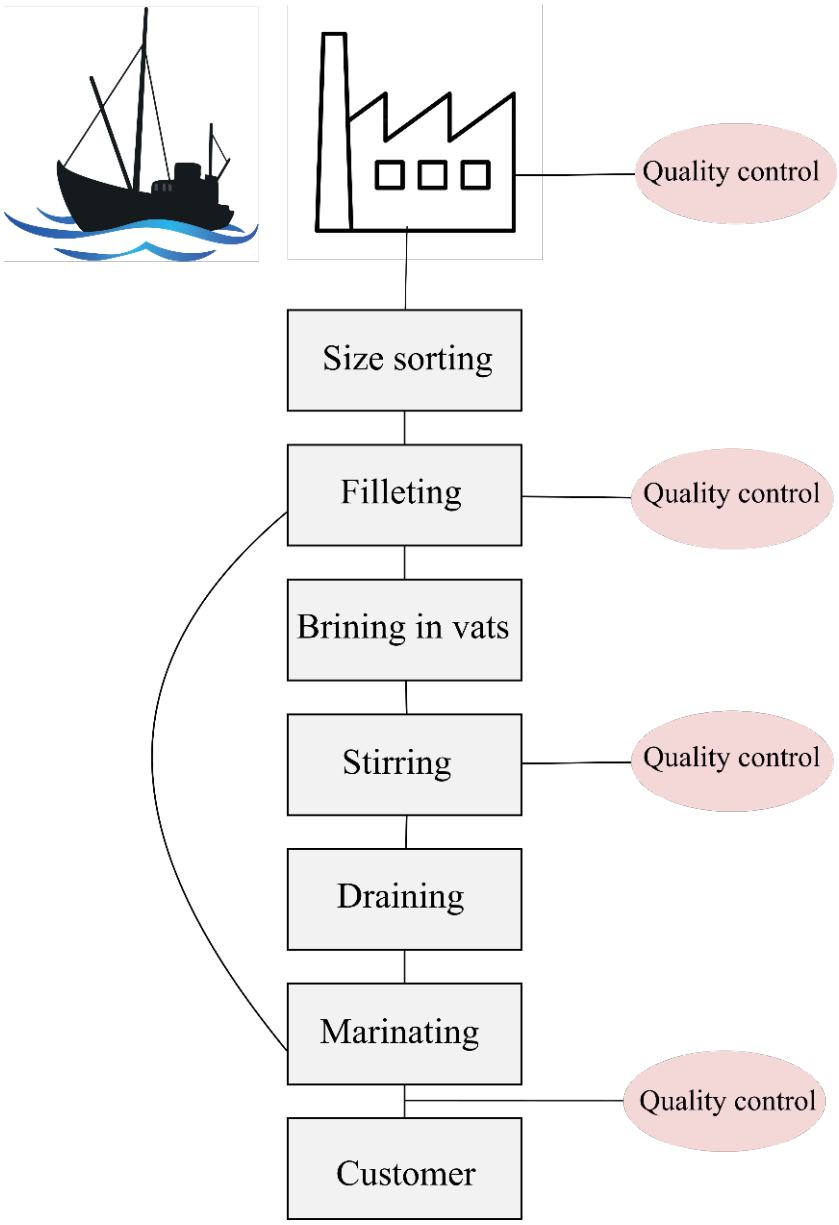

Figure 2.2: A typical Danish herring marinating process. The marinated herring products are sold as semi-manufactured products that are further processed before consumption.

This project deals with the marinating process of herring fillets similar to the Danish process. Figure 2.2 illustrates a typical Danish herring marinating process, which is described in the following. After arrival, the herrings are pumped from the large fishing vessel to the factory. The average fat content is determined in the beginning of each lot as part of the quality control in order to determine further processing. The herrings are sorted according to size, gutted, and filleted as butterfly fillets (kept together by the skin), as two separate fillets, or cut into smaller pieces. Typically, brining is carried out by immersion of fillets in brine (tap water and sodium chloride) in large vats and stored at low temperatures. Stirring and storage time depends on fat content, size, and on the type of brining process. Low brine-to-fish ratios (1:1) are commonly used in most industrial processes resulting in changes of the brine concentration during the brining process. The subsequent marinating process is carried out in large barrels, where the pre-brined herring fillets are submerged in a solution consisting of salt and acetic acid. Sometimes, the intermediate brining step is left out and the herring fillets are directly submerged into the marinade. The storage time depends on the concentration of the solutes and the herring is often stored for a minimum of 35-40 days and often for longer periods. The marinated herring products are sold as semi-manufactured products that are further processed before consumption. Quality control is conducted in the beginning, during processing and for the final products.

2.3 Effect of salt and acetic acid on fish muscle proteins

The fish muscle properties changes during the salting and marinating process because of the interaction of salt and acetic acid with the protein matrix (Duyar & Eke, 2009). The fish muscle consist of muscle fibres, which are lying parallel to the body and crossed by sheets of connective tissue that forms fibre segments also known as myotomes. The proteins consists mainly of the myofibrillar proteins, the sarcoplasmic proteins, and the connective tissue proteins (Careche & Barroso, 2009). During brining of herring fillets, salt penetrates the fish muscle and dissolves in the water associated with proteins. Depending on the salt concentration in the brine, swelling or shrinking of myofibrils occur and alter the distribution of water within the muscle. At low salt concentrations around 1 M (~5.8 % salt) the myofibrillar proteins are solubilized and maximum swelling of the proteins occur (Gallart-Jornet et al. 2007a; Thorarinsdottir et al. 2011; Erikson et al. 2006), which causes water to be more tightly bound to the myosin micelles (Duerr et al., 1952). The Cl^- ions bind to the actin and myosin filaments increasing the negative charges of the proteins, which increases the space between the filaments causing the muscle swelling as illustrated in Figure 2.3 (Offer & Trinick, 1983; Schmidt, Carciofi, & Laurindo, 2008). This

results in salt and water uptake by the muscle and diffusion of the soluble proteins and non-protein nitrogen compounds in to the surrounding brine (Szymczak & Kołakowski, 2012).

At higher salt concentrations (>1 M) the protein solubility decreases and aggregation of proteins occur, which leads to a decreased water holding capacity. The water is therefore released from the muscle to the surrounding brine leading to muscle shrinkage (Barat, Rodrıguez-Barona, Andrés, & Fito, 2003; Duerr & Dyer, 1952). When the protein solubility decreases, it leads to less leakage of protein and non-protein fractions to the surrounding brine.

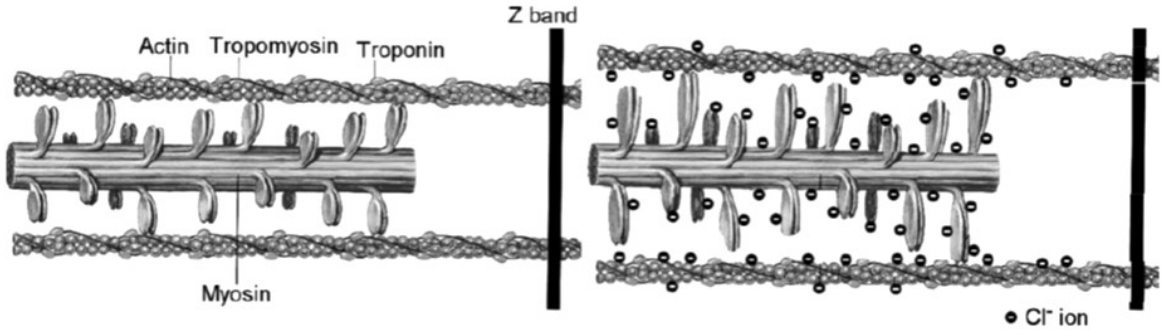

Figure 2.3: Illustration of the formation of complex Cl^--myofibrillar proteins increasing the matrix potential of the meat. Figure obtained from Schmidt et al., (2008).

In the marinating procedure, the acetic acid diffuses into the herring muscle. This lowers the pH and causes protein denaturation and lower water absorption (Szymczak, 2011). If only acetic acid is present in the marinade the pH of the muscle is on the acidic side of the isoelectric point. This leads to electrostatic repulsion causing an “open” structure and increased water holding. However, in the presence of salt the repulsive charges are shielded from one another causing a decreased water holding capacity. The extent of the effect of both salt and acid is therefore related to their concentration (Rodger et al., 1984). Using only acetic acid in the manufacturing process would result in soft fillets and for that reason salt is needed (McLay, 1971).

2.4 Effect of salt and acid on weight change

Fish muscle proteins are highly affected by the brining and the subsequent marinating process. The multiple transport of water, salt, acetic acid, salt-soluble proteins, smaller peptides and other nitrogenous compounds between the herring and the surrounding media therefore results in a weight change (Rodger et al., 1984; Szymczak, 2011; Szymczak & Kołakowski, 2012). How much the weight change, depends on the concentration of salt and acetic acid present in the

brine and marinade. Low brine-to-fish ratios such as 1:1 are often used in industrial set-up, whereby the transfer of salt and acetic acid into the fish consequently decreases the concentration of the brine/marinade. This again, affects the final concentration of salt and acetic acid in the fish and therefore the weight yield (Rodger et al. 1984; Capaccioni et al. 2011). A weight decrease have been observed for cod and salmon when brined in 25 % salt using the brine-to-fish ratio 3:1 (Gallart-Jornet et al., 2007a), however, for herring fillets brined in the same salt concentration using the brine-to-fish ratio of 1:1 a continuous weight increase was seen during the brining process (Birkeland et al., 2005).

In the subsequent marinating process, where acetic acid is present, water is diffusing out of the fish muscle resulting in decreased weight. It has been shown that the weight decreases with increasing acid concentration for both tunny fish (Topuz, 2016) and herring fillets (Szymczak, Kołakowski, & Felisiak, 2015). Achieving high product yield, which is defined as the weight of the herring after processing, without compromising product safety and consumer satisfaction is an important aspect of a profitable industrial production.

2.5 Effect of salt and acid on safety

Pickled herring products are not heat treated, but if the herring processing complies with the concept of a semi-preserved fish product with salt concentration >6 % in the water phase, pH values <5.0, and are stored ≤10 °C presence of typical environmental pathogenic bacteria such as *Listeria monocytogenes*, *Clostridium botulinum*, *Clostridium perfringens* and *Bacillus spp*. are not considered a significant hazard. Presence of bacterial pathogens such as *Salmonella spp*., *Shigella spp*., *E. coli*, *Campylobacter* and *Staphylococcus aureus* is the result of fecal transmission from human/animal reservoir or poor process hygiene. Contamination by these pathogens is a potential hazard, but can be prevented by good manufacturing practices (GMP) and good hygiene practices (GHP) (Huss, Ababouch, Gram et al., 2004).

Wild caught fish are considered at risk of containing viable parasites and the *anisakis simplex* larvae is associated with herring (EFSA Panel on Biological Hazards, 2010). The current EC regulation 853/2004 requires freezing at -20 °C for minimum 24 h of "marinated and/or salted fishery products, if the processing is insufficient to destroy nematode larvae". Freezing must be applied to either the raw product or the treated product (EC, 2004). However, semi-preservative products like salted and pickled herrings are not always produced from frozen raw material because freezing is known to affect the structural proteins and consequently affect product yield.

Recommendations for inactivation or killing of nematodes in herring by use of the marinating procedure are given in various documents. These include the Opinion of EFSA on parasites in fishery products (EFSA 2010) and the FAO Fisheries Technical Paper 444 (Huss, Ababouch, Gram et al., 2004), which are both based on research conducted by Karl et al., (1995). They found that with a minimum acid concentration of 2 % in the fish water phase the survival time of the *Anisakis simplex* decreased with increasing salt concentration in the water phase: 4-5 % salt > 17 weeks of storage (3 °C), 6-7 % salt 10-12 weeks of storage and 8-9 % salt 5-6 weeks of storage (Karl et al., 1995). They also state that salt is the most important factor in the inactivation of nematodes whereas (Meyer, 1965) reported that the most important factor in preservation of herring is acetic acid.

2.6 Texture

Texture is an important quality parameter for the fish processing industry as well as for the consumers. Texture of the raw material is important in relation to the handling during processing and the texture of the final products are important for the consumers. Texture of fish muscle depends on several factors; internal factors such as age, size and nutritional state of the fish and external factors such as storage conditions and processing (Hyldig & Nielsen, 2001). The main components related to fish texture are considered to be the connective tissue and muscle fibres (Delbarre-Ladrat et al., 2006; Dunajski, 1979). The texture is dependent on ante-mortem factors (such as size, age and physiological status) and post-mortem factors (such as rigor mortis, pH and proteolysis) (Dunajski, 1979). Soft flesh and gaping is often found during cold storage of fresh fish. A degradation of the myofibrillar proteins have been associated with textural changes in fresh fish (Hyldig & Nielsen, 2001) as well as a reduced strength and content of the connective tissue (Delbarre-Ladrat et al., 2006; Mørkøre & Austreng, 2004). Gaping refers to the gapes or slits in the connective tissue between the muscle segments or the individual myofibers. Gaping can occur when the fish muscle is still attached to the skeleton when rigor mortis takes place and the muscle is not free to shrink (Dunajski, 1979).

Fat content and the distribution of fats in the fish fillet may also affect the texture of the fish meat (Sigurgisladottir, ØTorrissen, Lie, Thomassen, & Hafsteinsson, 1997). Decreasing hardness values from front to tail has been reported for salmon fillets both for samples with natural fillet thickness as well as for standardized fillet thickness (Jonsson, Sigurgisladottir, Hafsteinsson, & Kristbergsson, 2001; Sigurgisladottir et al., 1999). Studies mapping the fat and water distribution in salmon fillets showed that the water content increased while the fat content decreased towards to the tail region for the dorsal part of the muscle (upper mass) (Segtnan et

al., 2009; Zhu, Zhang, Shao, He, & Ngadi, 2014). This change in water and fat content may explain decreased hardness towards the tail (Jonsson et al., 2001; Sigurgisladottir et al., 1999).

Processing of fish using salt and acetic acid affects the structure and conformation of the myofibrillar and connective tissue proteins (Dunajski, 1979). It also affects the water holding capacity. When the water content in the muscle decreases an increase in toughness is seen (Dunajski, 1979). Rodger et al. (1984) found that increasing salt and acid concentration in the herring marinating process decreased the moisture content causing dehydration of the fish muscle, which lead to an increase in the texture parameter. Dry salting and brining of cod using a saturated salt solution (excess brine) caused a hardening of the fish muscle because of denaturing of proteins (Barat et al., 2003).

Using frozen raw material compared to fresh may lead to differences in the final product yield as well as textural properties. Frozen storage of fish disrupts the muscle structure causing denaturation of the proteins. This leads to protein aggregation and a subsequent drip loss after the fish is thawed. The change in muscle structure can also lead to changes in salt and acid diffusivities (Deng, 1977), and thereby affect the weight change during processing (Szymczak, Kołakowski, & Felisiak, 2012). The denaturation of proteins caused by freezing is also believed to change the textural properties causing a toughening of the muscle (Cheng, Sun, Han, & Zeng, 2014; Dunajski, 1979; Hyldig & Nielsen, 2001). The textural changes occurring during frozen storage are also affected by storage time and temperature (Careche & Barroso, 2009).

Several methods for texture evaluation of fish exist, both sensory and instrumental methods. Choosing the most relevant method to evaluate the textural quality of the raw material, as well as changes occurring during processing, is therefore of great importance. Texture of whole fish fillets is difficult to assess due to the lack of uniform structure and within the same batch, there can be large variation from fish to fish. Moreover, the texture may vary along the fillet, which adds difficulty to the measurement (Careche & Barroso, 2009). Studies have shown that testing parameters such as type of probe, sampling speed, sample thickness as well as temperature affect the results of the instrumental texture analysis (Hyldig & Nielsen, 2001). In the present PhD thesis, only the methods used are described. A more extensive review of both sensory and instrumental methods for fish texture can be found in Hyldig and Nielsen (2001).

Compression tests can include one or two successive compressions, where using the latter corresponds to the Texture Profile Analysis (TPA) (Hyldig & Nielsen, 2001), also associated with a “two bite” test. Compression tests are often less destructive compared to methods where the fish muscle is penetrated or cut (Dunajski, 1979). The compression can be performed by compressing the sample to a fixed distance, percentage deformation or at a set force (Careche &

Barroso, 2009). Texture parameters like hardness and fracturability can be determined by compressing the sample once. If double compression is used instead, several parameters can be determined at once; cohesiveness, elasticity, adhesiveness, chewiness, gumminess and springiness (Hyldig & Nielsen, 2001). According to Careche and Barroso (2009) a true compression test requires that the probe is larger than the sample. The samples should also be standardised, as the physical dimensions of the sample is one of the parameters that can affect the measurement. However, normalization of the samples can be very difficult because of the natural variation in fillet thickness. Texture is therefore measured directly on the fish fillets, usually using a sphere probe. This method is applied to resemble the "finger" method, which is often conducted in the industry and is less destructive than the "true" compression test (Careche & Barroso, 2009).

Evaluation of fish texture in the manufacturing process is conducted as a subjective assessment of firmness by touching the fish and visual inspection. The so called "finger method" has been applied in various industrial investigations and by pressing the finger on the flesh both the firmness and elasticity is evaluated (Sigurgisladottir et al., 1997). However, this method is highly subjective and methods that are independent of the person conducting the measurements are more desirable.

2.7 Transfer mechanisms

During the marinating process a simultaneous transfer of water, salt, acetic acid, soluble proteins, small peptides and other nitrogenous compounds occur. The changes in the muscle affects the chemical fluxes and the transfer of the different components are interdependent of each other (Thorarinsdottir, 2010). In addition, fat as well as the skin are also known to influence the salting process and be a limiting factor for salt and water diffusion. Fat can either replace the aqueous phase that serves as a path for transfer or acting as a physical barrier (Gallart-Jornet et al., 2007a; Rodger et al., 1984; Rørå, Furuhaug, Fjæra, & Skjervold, 2004).

In this project, the transport of water and salt is in focus. Understanding the salting kinetics is of great importance for industrial processes in order to develop new products and optimize existing processes conditions. Mathematical models can give a better understanding of the transport phenomenon, and a better control of process variables such as brine concentration and immersion time in order to obtain a determined salt and water content and ensure a safe product (Andreetta-Gorelkina, Gorelkin, & Rustad, 2016; Zhang, Xiong, Liu, Xu, & Zhao, 2011). Diffusion is commonly referred to as the main transfer mechanism when describing salting kinetics. The salt transfer is caused by the concentration gradients between the fish muscle and

the surrounding media, as the force driving the transport (Thorarinsdottir, 2010). In the beginning of a brining process, the salt transport takes place via a layer of salt solution covering the fish with a lower concentration of salt than the rest of the surrounding brine. This is because water is diffusing from the fish in the very beginning of the process with a greater speed than the salt intake (Voskresensky, 1965).

Generally, the diffusion of a solute into a solid material is described by Fick (1855) and the concentration profile can be determined by means of Fick's law. Fick's second law describes a non-steady state diffusion, where it relates the change in concentration over time with the change in flux. Fick's second law is presented in eq. 2.1:

$$\frac{\partial C_s}{\partial t} = D\frac{\partial^2 C_s}{\partial x^2} \qquad \left[\frac{kg}{kg \cdot s}\right] \qquad \text{(eq. 2.1)}$$

Where C_s is the salt concentration (kg·kg), D_s is the diffusion coefficient of ($m^2\ s^{-1}$), x is the position in the direction of diffusion (m), and t is the time (s). Different boundary conditions need to be applied in order to integrate Fick's law. For example considerations on the geometry of the body and if the concentration of the salting media changes over time or it is kept constant (Vestergaard, 2004).

The transport of water is governed by the water activity gradient, which is in function of the salt and water concentration ratio, and is affected by the salt-protein interactions in the fish muscle (Barat et al., 2003; Thorarinsdottir, 2010). The water flux in function of the water activity gradient (Bird, Stewart, & Lightfoot, 2007) is given by eq. 2.2:

$$J_w = -C_w \cdot D_{a_w}\frac{d\ \ln a_w}{dx} \qquad \left[\frac{kg \cdot m}{kg \cdot s}\right] \qquad \text{(eq. 2.2)}$$

Where J_w is the water mass flux ($kg \cdot m \cdot kg^{-1} \cdot s^{-1}$), C_w is the water concentration (kg·kg), D_{a_w} is a mobility constant ($m^2 \cdot s^{-1}$) and ln a_w is the natural logarithm to the water activity (-).
As water counter diffuses to equilibrate the activity gradient, it ends up eventually leaving or entering the system, leading to a volume reduction or increase.

No universal method exists for obtaining information about the distribution- or the diffusion coefficient of salt and moisture and the method most appropriate to apply depends on the food that is investigated. Diffusion coefficients are determined experimentally and by fitting to solutions of Fick's law. This is conducted either by measuring the average salt concentration over time (global concentration profiles) (Wang, Correia, and Tang, 1998; Wang, Tang, & Correia, 2000) or by measuring the concentration profiles at different times during salting (local

concentration profiles) (Vestergaard et al., 2007). Obtaining local concentration profiles makes it possible to study the distribution of salt and water inside the fish muscle and the change over the salting process. Local concentration profiles of salt have been investigated using a non-destructive method using ^{23}Na Magnetic Resonance Imaging (MRI) in meat (Vestergaard et al., 2007) and in salmon and cod (Gallart-Jornet et al., 2007a) and by destructive methods where the concentration profiles were quantified by slicing the fish samples for sardines (Boudhrioua, Djendoubi, Bellagha, & Kechaou, 2009).

2.8 Near infrared spectroscopy

NIR spectroscopy has successfully been implemented in the food industry as a fast quality control method both at-line, in-line and on-line. NIR spectroscopy is defined as the spectral region from 800 nm to 2500 nm and is based on vibrational modes of molecules mainly C-H, O-H, and N-H functional groups, which can be observed as overtones and combinations in the NIR spectrum (Huang, 2001; Svensson, Nielsen, & Bro, 2004). Because of broad overlapping peaks of different constituents, the method is considered as a nonspecific method, hence an indirect method. For that reason, NIR measurements have to be calibrated against a known chemical value (a reference) using mathematical methods. Near infrared spectroscopy is a useful analytical technique for different biological samples and works by measuring the amount of light absorbed by the sample as a function of the wavelength (Galvis-Sanchez et al., 2011).

Selection of the most appropriate measurement principle (transmission or reflectance) is based on the current process and product of interest. NIR is known to be sensitive to particle size in the sample as well as temperature changes. Temperature changes affect the NIR spectrum and can be seen as shifts of the O-H first and second overtone towards lower wavelength (higher frequency) (Cozzolino et al., 2007; Siesler, 2008). Temperature changes can occur during processing, but is not a problem for at-line NIR measurements as the sample have time to equilibrate to room temperature. However, during on-line measurements the probe is subjected to the actual temperature of the product during processing, which can be problematic. Arnold, Gaensakoo, & Harvey (1962) observed a temperature increase in a fermentation process resulting in highly complex spectral changes that were not predictable. The authors suggested that the change in temperature could be incorporated in the model as a variable, but most often it is desired to keep a constant temperature when using NIR.

Pre-processing of NIR spectra is conducted with the goal of removing physical effects in order to improve the multivariate regression. These effects arise from differences in parameters such

as sample size, scatter from particles, and molecular interactions. The choice of pre-processing method depends on the specific goal of the model (both the physical and the chemical changes in the sample could be of interest) and the type of sample. Therefore, one should carefully study the NIR spectra and evaluate the effect of the pre-processing in relation to the aim of the modelling. The most widely used pre-processing techniques used for NIR data can be divided into two categories; spectral derivatives (Typically Savitzky-Golay and Norris-Williams) and scatter correction (typically Standard Normal Variate (SNV) and Multiple Scatter Correction (MSC)) (Rinnan, Berg, & Engelsen, 2009).

NIR has successfully been applied for many types of food products with various applications. A review presenting some of the applications to food is given in Porep, Kammerer, & Carle (2015). Relevant for this project is the application of NIR to determine salt concentration in food products and aqueous solutions. NIR has been used to determine salt concentrations in meat (Begley, Lanza, Norris, & Hruschka, 1984), cod (Galvis-Sanchez et al., 2011), cured salmon roe (Huang, 2001) and hot smoked salmon (Lin, Cavinato, Huang, & Rasco, 2003) and for aqueous solutions (Hirschfeld, 1985; Lin & Brown, 1993).

2.9 Chemometrics

Application of NIR spectroscopy in a food process where several wavelengths are measured generates vast amounts of data. To analyze this type of data chemometrics can be used as it enables extraction of information concerning the carbohydrates, proteins, and fat in the food sample. Principal Component Analysis (PCA) is an explorative tool that creates a representation of the data in a new space covered by the principal components (PC's). The first PC represents the largest variation in data and the second PC represents the second largest variation (the second PC is orthogonal to the first PC) and so forth for the higher order components. PCA is often used as a first step in the data analysis of multivariate data because of its ability to reduce the complexity and enhance the interpretation of the sources of variation (Wold, Esbensen, & Geladi, 1987). In short, the original data matrix is decomposed into a structured part and a noise (residual) part eq. 2.3:

$$X = TP' + E \qquad \text{(Eq. 2.3)}$$

Where X is the data matrix (original data), TP' is the matrix product (assuming that two or more components are calculated; otherwise the equation would the vector product of scores t and loading p) representing the model with a given number of PC's. T, P and E are the scores,

loadings and residual matrix, respectively. The scores (T) contain information about the samples and the loadings (P) contain information of the variables and together they describe the underlying structure of the data. The residuals (E) contain the remaining information of X that was not described by TP' and ideally do not carry any systematic variation, but noise only. PCA is widely used for visualization of data and for outlier detection where especially the residuals are investigated further (Wold et al., 1987). Outliers have great impact on the calculation of the calibration models as PCA is a least squares method. Assessment of outliers is often conducted in the first initial PC plots (Wold, Sjöström, & Eriksson, 2001).

Partial Least Squares (PLS) regression is a linear regression method developed to maximize the covariance between the data set, X, and the response variable, y. The information in y is used for the decomposition of X in order to reduce the large variation in X that is not relevant to y. In this case y represents an observation from a chemical analysis for each sample. PLS is used to build a calibration model, where a reference value can be predicted in new samples. A central point in the calibration of the PLS model is to determine the number of components to include in the model and this is often done based on evaluation of validation errors (Wold et al., 2001). Using too many factors the data will be over fitted and the models might include non-generic parts (strongly calibration data dependent) resulting in poor predictions of new samples. On the other hand, using too few factors then the components do not include all the relevant information of the spectral data to create reliable models (Porep et al., 2015). Validation of the models predictive performance for unknown samples needs to be evaluated. The root mean square error (RMSE) is an important criterion and is shown in eq. 2.4.

$$RMSE = \sqrt{\frac{\sum(y - \hat{y})^2}{n}} \qquad \text{(eq. 2.4)}$$

Where y and $\hat{y}$ represent the measured reference and the predicted value, respectively, and n is the number of samples. The unit is the same as the original measurements and RMSE should be as low as possible in order to obtain an acceptable calibration model (Porep et al., 2015). The terms RMSEC and RMSECV are used to describe the internal calibration error, where the first term is an estimate of the prediction error where the calibration model is tested directly on the calibration data and is rather an estimate of the model error than the prediction error. RMSCV is the cross-validated error and is also based on the calibration data only. RMSEP is the root mean square error of prediction using external validation. This means that an independent validation set or test set is used to test the model performance. In order to perform external

validation the data set can be split into a calibration set and a test set. This however, has some disadvantages as the removal of samples for validation set can have consequences for the quality of the calibration model. This is also not considered as a true independent test set as the samples originate from data set. True independent test set are more difficult to obtain and could be samples from another time of year or another experimental batch (Porep et al., 2015).

Chapter 3: **Experimental work**

This chapter presents the experimental work related to the study of the process (3.1), measuring the concentration development (3.2) and process control using NIR (3.3).

The work is divided into three main parts shown in Figure 3.1. The first part describes the experimental approach to investigating the changes in herring fillets during processing and storage. The second part describes the development of local and global concentration profiles. Lastly, the third part describes the application of NIR spectroscopy and multivariate data analysis for non-destructive measurements of salt during processing.

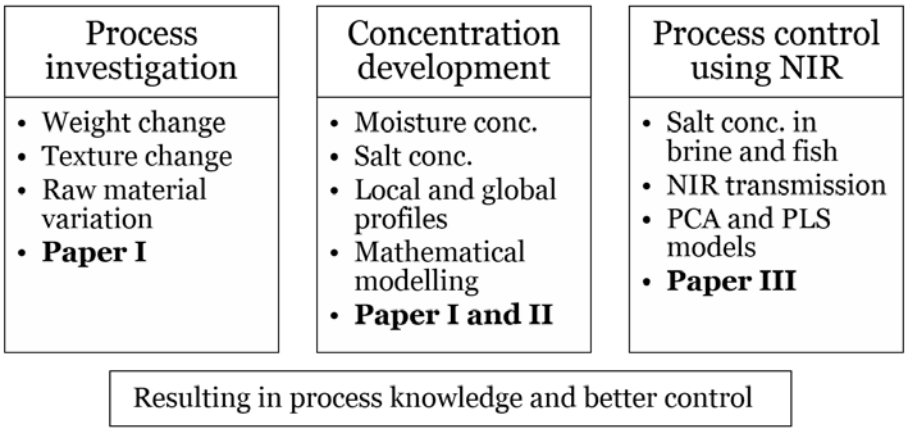

Figure 3.1: Overview of the experimental design divided into three parts: 1) investigations of the herring marinating process, 2) studying the salt and water concentration development in local and global measuring for modelling, and 3) the application of NIR spectroscopy in process control.

3.1 Investigations of the process

The focus of the experiments has been to investigate the effect of different brining and marinating conditions on the weight change of individual herring fillets. Experiments were conducted on butterfly fillets, where one fillet was used for chemical analysis of the raw material and the other fillet was used for the process experiments. Using double fillets makes it possible to study the relation between the fat content and the process parameters and the effect on the

final product yield on individual fillets. Preliminary studies showed that the two fillet sides were not significantly different from each other in relation to the fat content and was therefore considered alike for the rest of the project. Fresh herring fillets were used for most of the experiments, because fresh herrings are mostly used for industrial processing in Denmark. Herring fillets were obtained from Skagerak Pelagic A/S in Skagen, Denmark, mainly caught in the North Sea.

One of the main issues in the manufacturing of pickled herring products is the varying product yield caused by the varying the raw material (i.e. the fat content) and the differences in storage time, which is why these studies were initiated. The experiments were conducted in the timespan from 2014 to 2017, which made it possible to investigate batch variation as well as yearly differences of the raw material (i.e. the fat content). A brine-to-fish ratio of 1:1 was used in the brining and marinating experiments if not stated otherwise. This ratio is commonly used in a typical Danish industry. The experiments can be divided into two types; brining and the combined process consisting of brining followed by marinating.

Initially, marinating experiments were conducted in single plastic containers with lids. The fillet was placed and the brine/marinade was added. Using this type of containers made it difficult to obtain the wanted fish-to-brine ratios and full coverage of the fillets as they floated to the surface. This setup is also very far from the industrial process, where app. 80 kg of herring fillets are marinated in each barrel. It was therefore chosen to tag each herring fillet using a tagging gun in order to keep track of the individual herring fillet during processing in larger buckets. All storage experiments were carried out at 2 °C.

3.1.1 Herring brining process

Brining of herring fillets was carried out in order to study the flux of salt and water and the effect on the fillet weight over the course of 24 hours, which is similar to the storage time for the intermediate brining process in the industry. Frequent sampling (every hour) of brine and fillets was necessary, since great changes in the salt and water transfer occur within this timeframe (Birkeland et al., 2005).

Measurements

Salt, moisture, and weight were measured throughout the storage period and is described in further detail in paper I. Furthermore, NIR and protein were measured on brine samples to study the effect of initial brine concentration on the diffusion of protein to the brine and to follow process dynamics using NIR spectroscopy.

Figure 3.2a shows a dispersion of the samples in relation to the weight change, which is most likely caused by the natural biological variation between the herring fillets. It is believed that the biological variation will overshadow the "true" weight change in the fillets and for that reason; the same fillets were weighed during the brining process (Figure 3.2b).

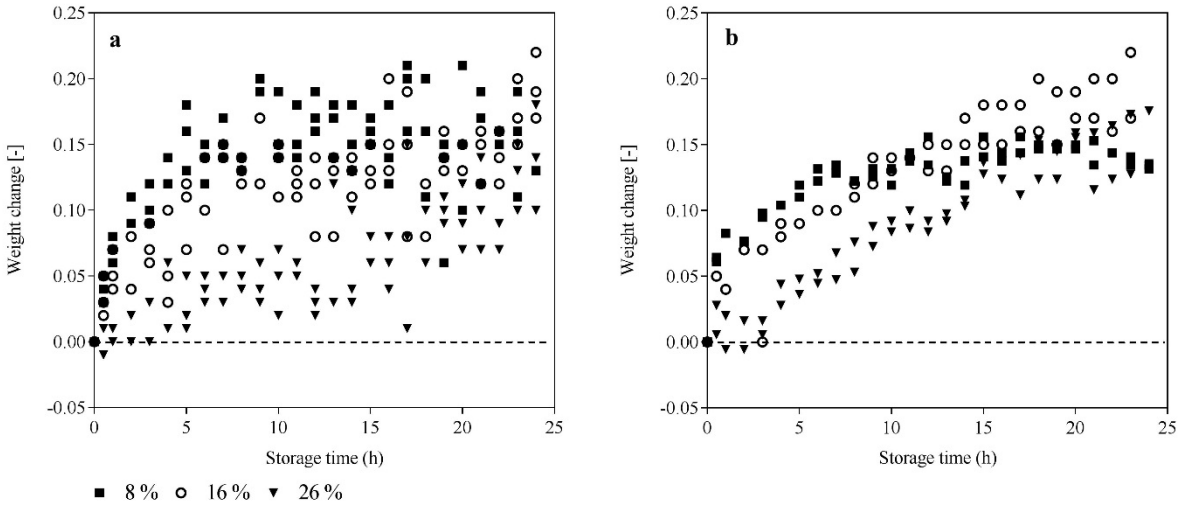

Figure 3.2: The fillet weight change during a 24 h herring brining experiment using 8 %, 16 %, and 26 % (w/w) salt brine in the brine-to-fish ratio 1:1, where different herring fillets are weighed (a) and the same herring fillets are weighed during the process (b).

3.1.2 Herring brining and marinating process

Several herring marinating experiments were conducted during this PhD project to study the effect of the intermediate brining process and the use of different acetic acid concentration on the weight change during processing. The storage time was varied to investigate the changes in weight occurring in the beginning of storage and during prolonged storage up to a year. Differences between using fresh or frozen raw material for the marinating process as well as the textural changes during processing was also investigated. Studies of the intermediate brining step in the marinating process was conducted because little information exists about this step and the effect on product yield after marinating. The effect of marinating on different quality properties has been studied for different fish species, however, the studies did not include salt brining as an intermediate step in the marinating process (Baygar, Alparslan, & Kaplan, 2012; Szymczak & Kołakowski, 2012; Szymczak et al., 2012; Topuz, 2016).

Measurements

The weight of each fillet was registered during the brining and marinating process. The fat content was measured on the raw fillets to investigate the correlation between the fat content and the weight change during processing (further details are given in paper I). Two approaches to texture analysis were used to study the quality changes in the fillets during processing as well as the difference between using fresh and frozen raw material:

1. By Texture Profile Analysis (TPA), evaluating several parameters, which include sample preparation before measuring.

2. Compression test evaluating the parameter "hardness" measured directly on the fillet with no sample preparation.

Further details of the measurements are given at the end of this chapter. Preliminary texture studies on fresh herring fillets, using the first approach, indicate that the sampling position may be an important factor that affects the result of the texture analysis (Figure 3.3). Fresh herring fillets were sampled at Skagerak Pelagic A/S and sorted into two categories: "Soft" and "Firm" by subjective evaluation. Maximum force was determined at four positons on each fillet using with a constant penetration depth of 2 mm in order to achieve a surface measure on the firmness relative to a 10 % compression.

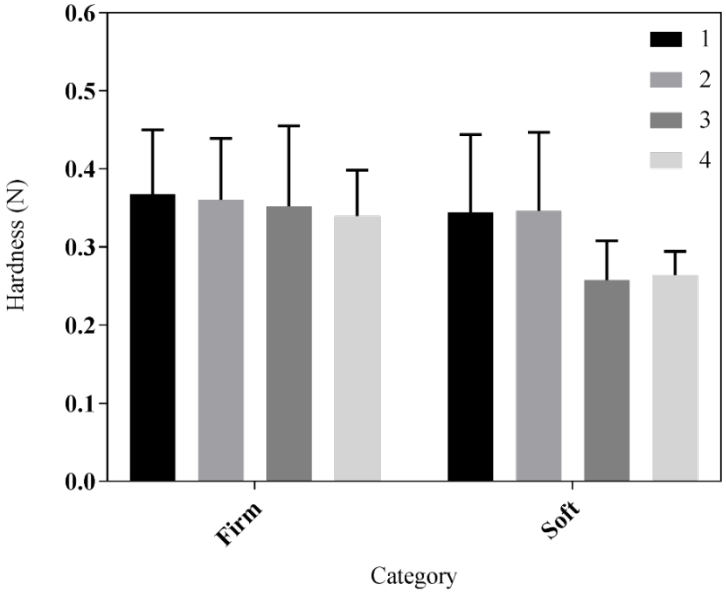

Figure 3.3: Compression test on fresh herring fillets measured with a constant penetration depth of 2 mm using a sphere at four locations from the head to the tail. Samples were naturally (not equally) thick. Data show mean and standard deviation of four fish. Fresh herring fillets were obtained from Skagerak Pelagic, Skagen, Denmark, sorted in soft (n=5) and firm (n=10) category.

A tendency of decreasing hardness values toward the tail region was observed (from position 1 to 4); however, it was more pronounced for the “Soft” category of fillets. Lower hardness values were seen at position 3 and 4 for the “Soft” category of fillets, which could be due to a more pronounced gaping structure of the muscle at the tail region resulting in the lower force applied for compression. For fillets belonging to the “Firm” category not much difference was found in the force applied at the four sampling positions, which was similar to the results obtained by Nielsen, Hyldig, Nielsen, & Nielsen (2005b). The similar hardness values obtained along the fillets belonging to the “Firm” category could be explained by the even distribution of fat and water along the fillet (Elmasry & Wold, 2008). Depending on the raw material quality, it may not be appropriate to use average values of the hardness values obtained on the fillet as a representative measure of the textural quality for the entire fillet.

Statistical analyses were performed using GraphPad Prism 7 multiple t-test to find significant ($P<0.05$) differences between the mean values of the groups at individual times. As correlation test the One-tailed Pearson Correlation ($\alpha=0.05$) was used. Methods that are not described in the papers are described in further detail at the end of this chapter.

3.2 Measuring the concentration development

The salt and water transfer during salting of herring was studied both by global and local measuring. Development of the model for the global concentration development during brining varying is described in further detail in paper I, where the aim was to provide a mathematical tool for practical application in the industry. The model was based on the development of the average concentration of salt in the fillet with a finite amount of salt present. As stated in the Background (section 2.3 and 2.7) the transport of salt in the herring muscle is influenced by several factors e.g. the salting method as well as the changes in the muscle during the salting process. For that reason, it was decided to obtain local concentration profiles to study the coupled moisture and salt transfer.

The salt and moisture transfer in the herring muscle was investigated using the time-dependent concentration-distance in order to understand the distribution of the diffusing substances within the products. The obtained diffusivities would be more reliable than those determined from the change in the average content of the diffusing substances over time (Boudhrioua et al., 2009). Local concentration profiles of the salt distribution in fish has been obtained for herring fillets by Rodger et al., (1984), where they describe the change in salt distribution inside the fillet over time and at different temperatures. For salmon and cod the local concentrations were found using the non-destructive method ^{23}Na MRI (Gallart-Jornet et al., 2007a), where the

authors describe the difference in salt diffusion in fatty and lean fish. However, the coupled transfer of moisture inside the fillet were not described. Moreover, these authors did not use the local concentration profiles to determine the diffusion coefficient of salt.

Our studies aimed to investigate the coupled transfer of salt and moisture in herring fillets when subjected to different salting conditions. The concentration profiles were obtained simply by cutting the fish into thin slices using a freezing microtome. The method is described in further detail in paper II.

Measurements

The height of each slice was measured using a Texture analyzer and salt and moisture content were determined for each slice. The benefit of using this method is that it is relatively inexpensive and does not require any special skills in data handling, however, the drawback is that it can be difficult to cut the samples in equal thickness. The samples varied naturally in thickness, which made it difficult to obtain slices of equal thickness and an equal number of slices from sample to sample. The slices were sometimes very thin, which introduced possible error in chemical measurements. The method to cut the samples using a freezing microtome was chosen because more uniform slices could be cut when the sample was frozen. In order to avoid salt and moisture changes during freezing of the muscle it was chosen to freeze the samples in liquid nitrogen and store them at -40 °C until further slicing. Slicing the samples in unfrozen state is more difficult because of its soft texture.

3.3 Process control using NIR spectroscopy

This study was initiated because salt is one of the key preservatives for pickled herring products, but it is also an important factor for the sensory characteristics (quality) of the product. Commonly salt determinations are conducted by the use of titration with silver nitrate, which is a time consuming and destructive method. For that reason, a faster and non-destructive method would be preferable, especially for the herring marinating industry. Moreover, it is known that variability between herring fillets occurs, especially in the fat content (Aidos et al., 2002; Lane et al., 2011; Nielsen et al., 2005a), and sampling of some fillets may not be appropriate for characterizing the whole batch. For that reason, sampling of the surrounding brine is very attractive and may be more representative and indeed more accessible than sampling the whole fish during processing. While, salt (NaCl) has no specific absorption band(s) in the NIR region, it is known that salt in solution causes changes in the height, width and position of the absorbance bands of water (Hirschfeld, 1985; Lin & Brown, 1993).

Experiments mimicking the industrial marinating process were included in the study, with variation of the brining and marinating process in relation to the concentration of solutes, storage time as well as the seasonal changes of the herring fillets. Sampling of brine/marinade was conducted after mixing using a whisk and two independent samples were extracted. Proper sampling of the marinade is important in order to attain a representative sample of the total volume because of the heterogeneity of the marinade due to diffusion of e.g. soluble proteins and water from the herring to the surrounding medium. A short description of the experimental sets is given:

1. Experimental set: **"April 2017"**: A total of seven marinating experiments were carried out for this data set varying the intermediate brining process in relation to brine concentration and storage time. Further details are given in paper III about the salting and marinating conditions used.

2. Experimental set: **"October 2016"**: A total of three brining experiments using 8 %, 16 % and 26 % salt concentration using the brine-to-fish (1:1) were carried out for this data set. The storage time was 24 hours where sampling (N=2) occurred every hour. Further details about the salting conditions are given in paper I.

3. Experimental set: **"December 2017"**: Three marinating experiments were carried out for this data set. Intermediate brining process was carried out for three days using 13 %, 16 % or 26 % (w/v) salt. Marinating was carried out under the same conditions for the three batches using 5 % salt and 6.7 % (w/v) acetic acid. Total marinating time was 26 days. N=30. Sampling occurred after 24 hours in the marinade, where it was assumed that equilibrium state was reached.

4. Experimental set: **"Old"**: The data set consists of three batches of two marinating experiments and one brining experiment. The first two batches were produced and delivered by Skagerak Pelagic A/S; the first batch was stored at least three years (unknown production date and process conditions) and the second batch was produced in April 2014. The last batch consists of herring fillets brined in 16 % (w/v) salt since December 2016, N=15.

5. Test set: "**February 2018"**: same experiment as the experiment "Dec2017", however, with a total of three months of storage. N=15.

Measurements

The marinade samples were centrifuged prior to conducting the NIR analysis to remove large tissue parts and to minimize the effect of different particle sizes on the NIR spectra. NIR spectra of brine/marinade samples were obtained using a Fourier Transform spectrometer (QFA-flex, Q-interline) using a cuvette with a light path length of 8 mm in transmission mode. Each sample was measured with the average of 128 scans (total duration approximately 40 sec.) over the spectral range of 1000 to 2500nm (10.000 to 4.000 cm^{-1}) with a spectral resolution of 16 cm^{-1}. A relatively large number of scans were used in this study and the acquisition time were around 40 sec. For industrial purposes, a lower acquisition time is preferable and the number of scans may be reduced.

All samples were brought to room temperature (duration of 1 h) before measuring, in order to avoid temperature induced shifts in the NIR spectra (Arnold et al., 1962). Air was used as the background for all spectra obtained and measured before the sampling. In this study, all spectra were mean centered, and several combinations of additional pre-treatment methods were explored. The best results were obtained by the application of SNV prior to mean centering. With applying SNV, the scattering is removed by normalizing each spectrum by the standard deviation (eq. 3.1):

$$X_{corr} = \frac{X_{org} - a_0}{a_1} \qquad \text{(Eq. 3.1)}$$

Where a_0 is the average sample spectrum and a_1 is the standard deviation of the sample spectrum. **Figure 3.4** demonstrates the SNV correction for representative NIR spectra of herring brine/marinade, where Figure 3.4a shows the raw NIR spectra and Figure 3.4b shows the SNV treated spectra.

Evaluating the effect of the spectral pre-treatment was done by visual inspection of spectral changes, by exploring PC scores (from a PCA model) of replicates to see how close they were to each other and by developing PLS models to evaluate the performance by comparing the validation error of the model. Inspection of outliers were also done in the plots of the PCA models and in the predicted versus measured plots of the PLS models. The number of Latent variables (LVs) included in the models was evaluated by inspecting the RMSECV, selecting the number of LVs where the curve for RMSECV flattened out or had a minimum.

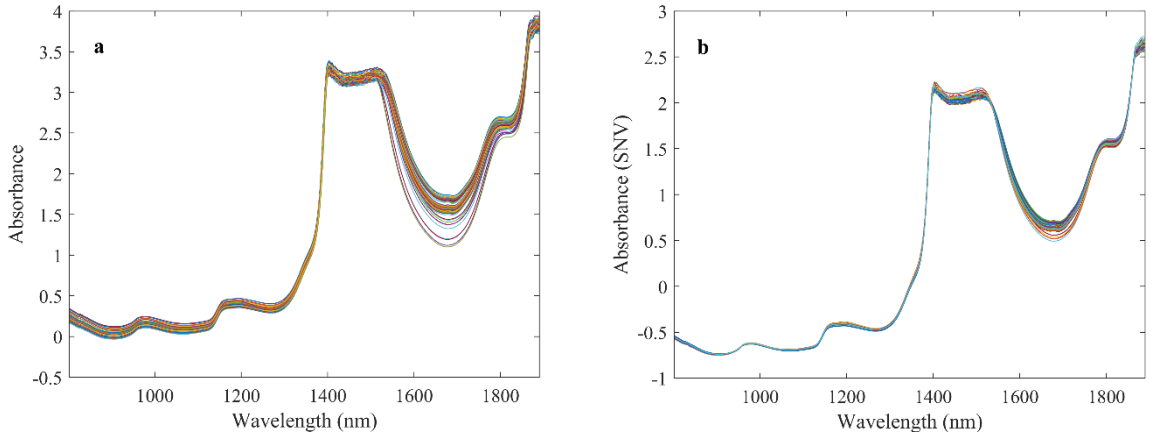

Figure 3.4: Visualization of the effect of pre-processing using SNV on NIR spectra showing the raw spectra (a) and SNV treated NIR spectra (b).

PLS models were developed to predict the salt concentration in the marinade and in the fish water phase. NIR is an indirect method and to determine the salt concentration in the fish from the brine samples makes the method a "double" indirect method. In order to use this approach the system needs to be in equilibrium. Three fillets were taken out for analysis at every time point and the average concentration of salt in the water phase of the three fillets was used for the PLS regression. Reference salt concentration values were measured on the corresponding brine/marinade samples as well as the salt and moisture content in the fish.

The PLS models were validated by segmented internal cross-validation (Venetian blinds with 10 splits and 4 samples in each), and external validation either by dividing the data set in a calibration and validation set or testing the model using an independent test set. RMSECV was used to evaluate the model predictions and is given in the same unit as the measured reference of salt in percentage or g/100 g. As correlation test between the PC scores and the chemical values the One-tailed Pearson Correlation (α=0.05) was used. All models were developed in the PLS_Toolbox (Eigenvector Research Inc., Wenatchee, WA) working under MATLAB 2016a v. 8.1.1 (The MathWorks, Natick, MA, USA)

Additional information of the methods used

Protein: The protein content of the brine was determined by the Kjeldahl method (Total N×6.25) according to AOAC methods (AOAC, 1995). In short, the method consists of a digestion of the sample converting the nitrogen to ammonia, then a distillation of the ammonia and lastly quantification of the ammonia by titration.

Texture: Texture was measured using a TA.XT2 Texture Analyzer (Stable Micro Systems, Surrey, England). Single compression: 10 % compression using a cylindrical probe (D=10 mm), test speed 1 mm/sec at four position equally dispersed down the fillet. TPA: Double compression test using a cylindrical probe (P10), 50 % compression, test speed 1mm/sec and 5 sec between the 2 compressions. Samples were prepared by cutting a 3x3 cm piece from the fillets 2 cm from the head part but the fillets kept the natural thickness. Several parameters from the TPA were calculated from the two curves and springiness was found to be the parameter to describe the change in the fillets during processing best. Samples were kept on ice to prior to measuring the texture to avoid texture differences due to temperature. Because the samples varied naturally in thickness, the hardness parameter was affected, which was also one of the reasons why it was decided to also study “springiness”. Springiness is defined as how well a product physically springs back after the first compression.

Chapter 4: **Results and discussion**

This chapter presents and discuss the main results obtained in this projects and is divided into two sections. The first section discuss the results relating to the salting process of herring fillets (4.1-4.3) and the second section discuss the results relating to the combined brining and marinating process (4.4-4.8)

Salting of herring using brine as an intermediate step in the herring marinating process is commonly applied in the manufacturing process in Denmark. The brining process is followed by a step where the herring fillets are submerged in a solution of salt and acetic acid. The first part of our investigations have been focused on the first step, salting of herring, alone, and the second part of the investigations have been focused on the combined process, where the fillets were initially brined and then marinated. For that reason, the results are presented related to either the salting of herring fillets or the full marinating process.

4.1 Salting of herring

During the brining process, the brine and herring fillets exchange salt and water. Therefore, both the brine and the herring fillets will undergo changes in salt concentration, water concentration and weight, thus directly influencing the safety and the yield of the final product. This was investigated in order to get a better understanding of the involved transport phenomena of salt and water and to gain a better control of the overall brining process. The results are presented in Paper I. The brining process is typically carried out using a brine-to-fish ratio of approximately 1:1 in the industrial manufacturing process (Birkeland et al., 2005). For that reason we studied the change in salt, water and weight of individual herring fillets using this ratio.

Salt uptake of the herring fillets is initiated due to the concentration gradient between the water phase of the herring muscle and the surrounding brine. The salt uptake continues until the salt concentration in the fish is equal to the concentration of the brine, which is illustrated in Figure 4.1a, where brine concentrations of 8 %, 16 % and 26 % were used. The increasing salt content in the fish during brining consequently decreases the water activity, which is illustrated in Figure 4.1b. This means that not only salt is transferred to the fish, but also water is moving from the brine into the fish in order to reestablish the water activity equilibrium, which is shown in Figure 4.2b. The water activity of the brine and the fish thus reaches the same level at

approximately the same time as the salt concentration is equalized. The final salt concentrations obtained in the herring fillets brined in 8 % and 16 % salt was below 9 %, which is reported to be a critical limit where the initial process of the water uptake changes (Duerr et al., 1952). This means that fillets with salt concentrations below 9 % experienced a water uptake during the brining process, whereas salt concentrations higher than 9 %, which was the case in fillets using the initial brine concentration of 26 %, lead to a decrease of the water content in the herring fillets in the beginning of the brining process shown in Figure 4.2b. The loss of water in the initial phase could be explained by protein denaturation mainly at the surface resulting in reduced water holding capacity (Gallart-Jornet et al., 2007a).

The coupled water and salt intake had a net impact on the fillet weight, which increased over time (Figure 4.2a). Equilibrium of salt and water concentration was reached for fillets during storage. The fillet weight seems only to have reached a steady state for fillets brined in 8 % salt whereas for fillets brined in 16 % and 26 % the weight increase throughout the storage period and equilibrium is possibly reach equilibrium later on (Barat, Rodrıguez-Barona, Andrés, & Fito, 2003; Birkeland et al., 2005).

During the experimental run fillets and brine were sampled every hour making it difficult to keep track of the brine-to-fish ratio. The possible change in the brine-to-fish ratios may have affected the salt and water concentration in the fish and consequently the weight change. However, as the fish samples were weighted and the same amount of brine were extracted the change in brine-to-fish ratio was limited to the best of our ability. Furthermore, more herring fillets were included in the experiments than actual needed for sampling in order to avoid ending with only a little amount of brine and few fillets left for the last couple of sampling points.

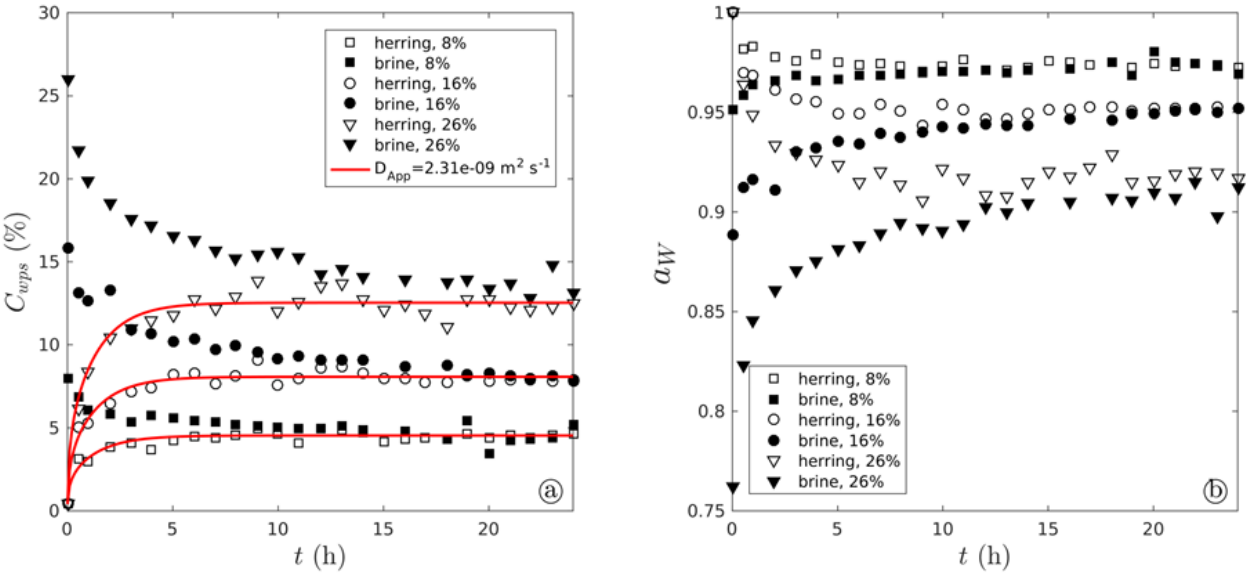

Figure 4.1: Change in the average salt concentration of the brine (n=2) and the fish water phase (WPS) (N=3) (a), where the discrete points represent the experimental data, while the solid lines represent the predictions of equation 4, calculated with α and the average D_{App} from Table 1 from Paper I, and the calculated water activity for the herring and the brine (b) during brining with 8 %, 16 % and 26 % (w/v) NaCl for 24 hours (Further details are given in Paper I).

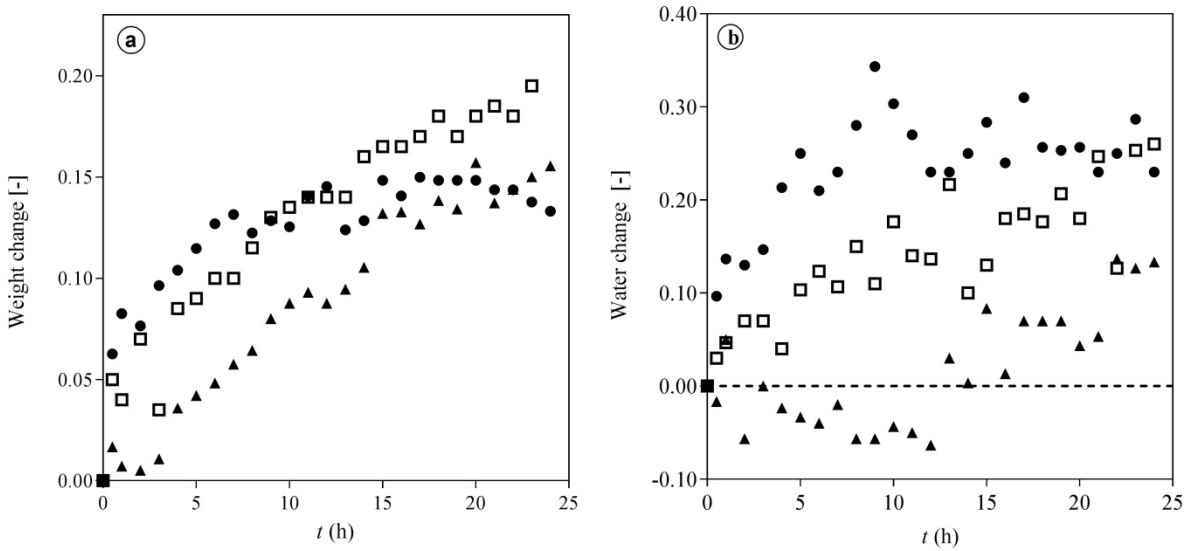

Figure 4.2: Change in fillet weight ($\Delta \boldsymbol{M_t}$) (a) and water content ($\Delta \boldsymbol{M_{w,t}}$) (b) during brining in 8% (●), 16% (□) and 26% (▲) NaCl solution (average values of N= 2) (shown in Paper I).

4.2 Concentration development

4.2.1 Global measuring

Knowledge of the salt and water transfer mechanisms is of great importance for the industry as it allows for estimating the process time to obtain a salt and water content that ensures a safe product. For that reason, a model describing the global salt concentration development in the herring (average salt concentration of the fillet) in function of time was conducted based on the average of the determined diffusion coefficients for three brining experiments. The predictions obtained by the model are shown in Figure 4.1a together with the experimentally determined salt concentration values. It was found that the average diffusion coefficient of salt, 2.31×10^{-9} m^2/s, for the three brine concentrations (8 %, 16 % and 26 %) could be used to describe the salt concentration development for any of the three cases.

Our results show that by increasing the salt concentration in the brine has an effect on the equilibrium concentration of the system, but not the process speed. These results are in agreement with the findings of Boudhrioua et al. (2009) who found that the salt diffusion was not affected by the concentration as well as the salting method for sardines. Nguyen et al. (2010) on the other hand found the diffusion coefficient to be decreasing as a function of increasing brine concentration in salting of cod. These two research studies mentioned above investigate the salt transfer using large quantities of brine or excess amount of dry salt in order to consider the brine concentration constant, which is one of the main differences between our study and these two studies.

In our study, we used a brine-to-fish ratio of 1:1 simulating industrial brining procedures, where the brine concentration cannot be considered constant and a direct comparison of the salt diffusion coefficients may not be possible. However, the diffusion coefficient found in this study is within the range of the reported values for sardines (9.80×10^{-10} to 1.20×10^{-8} m^2/s) by Boudhrioua et al. (2009). The disagreement considering the effect of brine concentration on the salt diffusion coefficients suggest that further studies are needed to clarify this issue. In order to simplify the model development, the focus was on the diffusion of salt, whereas the influence of water transport, which leads to weight changes in the fillets, was excluded. Global concentration profiles are commonly used to obtain information about the diffusion coefficient of salt, however, they do not provide any information about the local concentration gradient inside the herring fillet.

4.2.2 Local measuring

In order to achieve a better understanding of the system and the counter diffusion of salt and water, another study was carried out where local concentration profiles of herring fillets were obtained for both brining and dry salting of herring fillets. In this study, we use excess amount of salt to obtain a constant brine concentration and excess amount of dry salt in order to, contrary to the previous study, in order make the development of the model easier.

The results concerning the local concentration profiles are presented for brining of herring fillets in 26 % brine from 1 h to 48 h. The concentration of salt and water are expressed in $kg \cdot kg^{-1}$ because the driving force of the transfer is expressed in $kg \cdot kg^{-1}$. The concentration of salt and moisture were observed experimentally and is shown in Figure 4.3, while the water activity profiles were calculated and are displayed in Figure 4.4b. The salt and water concentration profiles obtained for dry salting and the brining in 16 % salt are shown in Paper II.

The concentration of either salt or water were lower in the skin interface compared to the muscle interface and were seen as asymmetrical distribution of salt and water around the centre of the herring fillet (Figure 4.3). This is especially noticeable at the beginning of the salting process and can be explained by that diffusion of salt primarily occurred from the muscle interface and a diffusion from the skin side was inhibited by the skin as well as the layer of fat beneath the skin (Rodger et al., 1984). Inspecting the profiles for salt concentration in the fish water phase and the profiles for the water activity, symmetrical profiles were seen with equal values at both interfaces after 48 h (purple) indicating the system is in a steady state (Figure 4.4a and b). However, the salt concentration in the herring water phase at 48 h is approximately 23 % and equilibrium of the salt concentration between the fish and the brine has not been fully reached. This can probably be explained by that no stirring was used for the brining experiments during storage.

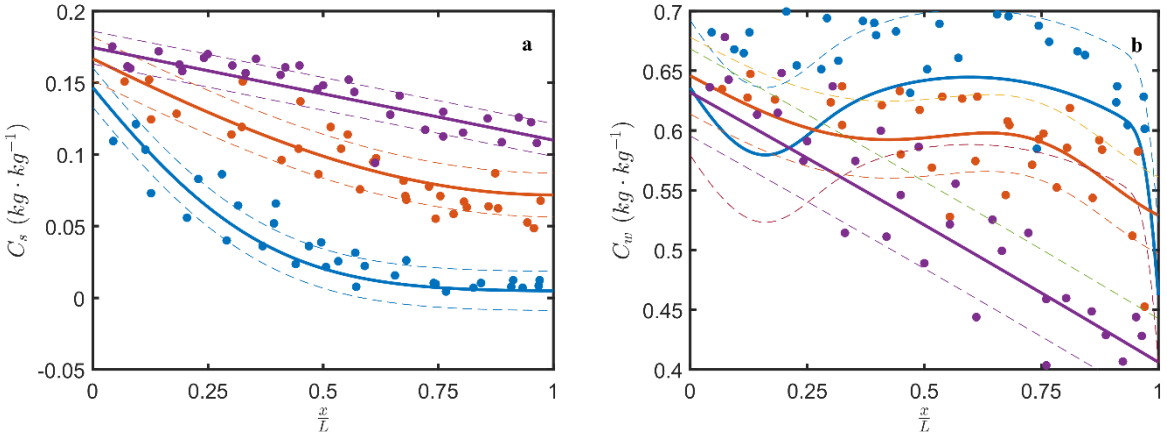

Figure 4.3 The salt concentration in the fish muscle (Kg·Kg^{-1}) (a) and the moisture concentration (Kg·Kg^{-1}) (b) versus the position (x/L=0 is the muscle interface and x/L=1 is the skin interface) for fillets brined in 26 % salt for 1h (blue), 6 h (orange) or 48 h (purple), (-) model fit, (--) RMSE. Further details are given in Paper II.

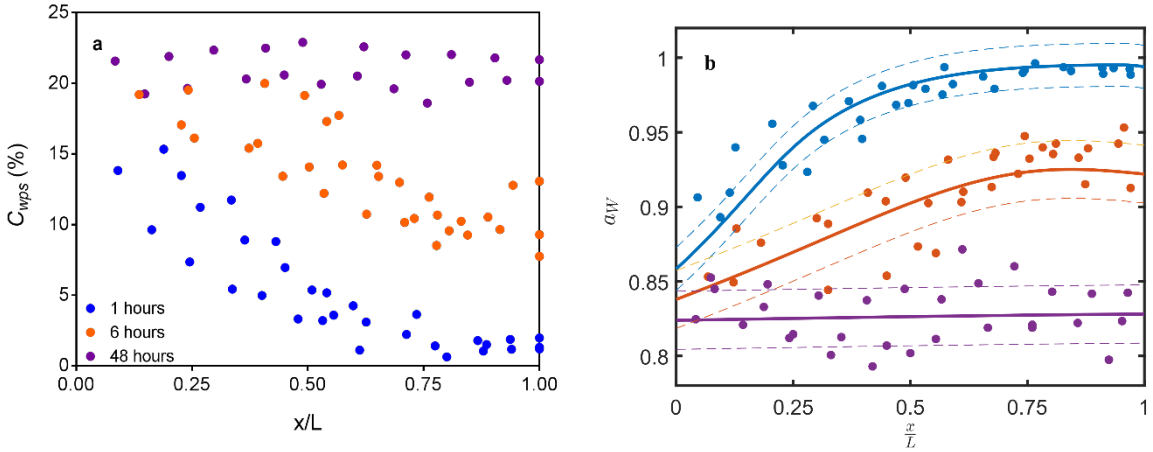

Figure 4.4: Concentration profiles for salt in the water phase (%) (a) and water activity (b) versus the position (x/L=0 is the muscle interface and x/L=1 is the skin interface) for fillets brined in 26 % salt for 1 hour (blue), 6 hour (orange) or 48 hour (purple) (-) model fit, (--) RMSE. Further details are given in Paper II.

The obtained diffusion coefficients were used to model the concentration profiles to predict the salt and moisture concentration and water activity profiles. Good model fits of the salt and water concentrations as well as water activity were obtained (Figure 4.3 and 4.4b, (-)) using the assumption of a constant diffusion coefficient. Poorer predictions of the water concentration profiles is observed in the beginning of the salting process, which is seen in Figure 4.3b at 1 h (blue) where the salt concentration in the fish is still low and the driving force is still large. This might be explained by the use of constant transport properties for the model development.

The determined diffusion coefficient for salt and moisture for 26 % salt brining were Ds= 15.2 × 10-1^{0} ^{m}2/^{s} and Daw$_{=2}$5.1 × 10-1^{0} ^{m}2/^{s}. These diffusivity values were similar to the values obtained for the dry salting process, however, slower diffusivity values for both salt and water, were obtained for brining at 16 % salt. According to Boudhrioua et al. (2009) the apparent diffusion coefficient for salt transfer was not dependent on the concentration of salt inside the fish or the salting method. However, they found that the moisture apparent diffusivities depended on the concentration of moisture inside the fillet, and with increasing moisture content in the fillets and increase in the moisture diffusivity was seen. However, their study differ from ours in relation to the direction of diffusion, which makes it difficult to compare their results directly with ours (Boudhrioua et al., 2009).

Several factors can influence the diffusion coefficients values for salt and water, which can be related to the experimental set-up and the modelling. The experimental set-up and how the diffusion coefficients are determined, vary between studies presented in the literature, which complicate the comparison of coefficients between studies. Some of the varying factors are mentioned and discussed.

In our study, the local concentration profiles were determined by destructive analysis by cutting the sample in thin slices and measure the moisture and salt content in each slice. This can be tricky to cut the samples in equal sized slices as the sample thickness vary because of the natural biological variation between the herring fillets. However, non-destructive methods do exist and the use of ^{23}Na MRI and ^{23}Na Nuclear Magnetic Resonance (NMR) has been used to study the salt diffusion and distribution in meat and in fish (Aursand et al., 2009; Bertram, Holdsworth, Whittaker, & Andersen, 2005). NMR is sensitive to water mobility and water's interaction with other molecules, whereas MRI is suitable to study the salt distribution in the fish, however the MRI equipment is expensive and the measurements are limited to a small sample size (Vestergaard, 2004).

The use of different salting conditions is also considered to affect diffusion coefficients. Swelling or shrinking can occur in the fish muscle during brining depending on the brine concentration,

and the change in muscle structure may highly change the speed of the salt and water transfer. A study conducted by Vestergaard, Andersen, & Adler-Nissen (2007), investigated the salt diffusion in meat using local concentration profiles. They found that the diffusion coefficient for salt differed inside the meat at the beginning of the salting process. The authors explained this by the fact that the salt transfer is not only governed by diffusion but also affected by the changes in the muscle structure (swelling) and counter-flow of water. The brine-to-fish ratio affects the concentration of the brine, and using low brine-to-fish ratios (1:1) results in a decreasing brine concentration as well as an increase in the salt concentration in the fish during the salting process. If a change in the salt flux occurs during processing it is difficult to uncover the reasons as it could be due to the increasing salt concentration in the fish or the fact that no more salt is available in the brine.

The dispersion of samples in the concentration profiles for the measured salt and water in the herring fillets (Figures 4.3 a and b) may be explained by the natural variation between the herrings related to the fat and moisture content (Nielsen et al., 2005a; Rodger et al., 1984). The amount of fat present in the fillets may also affect the transfer of salt and it has been suggested that a higher fat content can delay the salt transfer and take longer time to reach equilibrium concentration compared to lower fat content (Czerner & Yeannes, 2013). And in a comparative study of salt transfer in cod and salmon higher salt diffusion coefficient values were found for cod compared to salmon (Gallart-Jornet et al., 2007a).

4.3 Brining process monitoring using NIR spectroscopy

Most studies concerning the use of NIR Spectroscopy (NIRS) in food process analysis have dealt with characterization of the raw material as well as the final product. However, taking into account the consumers' requirement and the food regulation the use of models and process control helps ensuring food safety and food quality while keeping an efficient production and lowering production cost (Grassi & Alamprese, 2018). One of the complications though, is the variation of the raw materials, which in case of the fish industry is the variation of the fat content due to e.g. seasonal changes. With that in mind, it was found interesting to investigate the use of NIRS to study the process dynamics of brining of herring fillets.

The main idea with this study was to investigate the use of NIRS to gain information about the change in salt concentration during brining and to study the transfer of protein from the herring fillets to the brine during storage. The brine concentration decreased during brining of fillets with the use of 1:1 brine-to-fish ratio due to an uptake of salt in the herring fillets, which was

shown in Figure 4.1a. It was found that the total protein content increased in the brine during storage for all three brine concentrations (8 %, 16 % and 26 %), which is shown in Figure 4.5. No difference was found between the three brining conditions on the total protein content, which might be due to decrease in concentration of the brine during storage because of the low brine-to-fish ration of 1:1 and possibly the short storage time of 24 h.

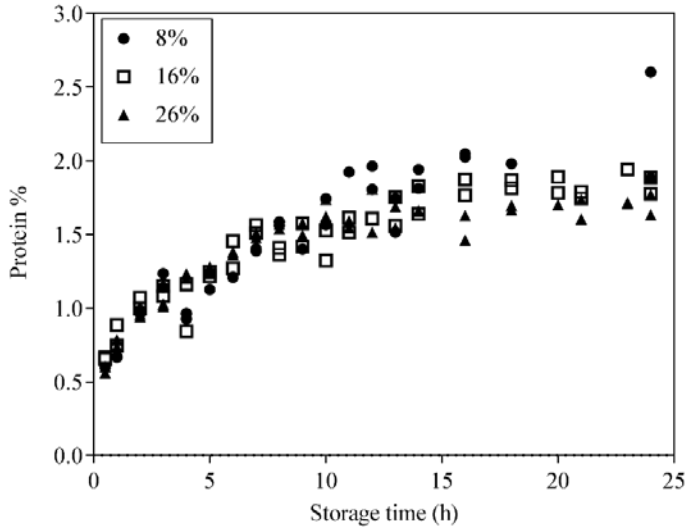

Figure 4.5: The protein content in brine samples during storage where herring fillets were brined in 8% (●), 16% (◘) or 26% (▲) salt using a brine-to-fish ratio of 1:1 for 24 h (N=2 per sampling point).

It was assumed that information about the changes in protein content could be found in the NIR spectra (Svensson et al., 2004). However, salt (NaCl) has no specific absorption band(s) in the NIR region, it is known that salt in solution causes changes in the height, width and position of the absorbance bands of water (Hirschfeld, 1985; Lin & Brown, 1993).

A PCA model was developed for the brine concentrations, 8 %, 16 % and 26 %. The first PC score values versus the process time for the three batches are shown in Figure 4.6a. A decreasing trend over time was observed for all batches, and the scores for the three batches differed from each other at all sampling times: the higher the initial brine concentration used the higher PC1 score values. Although NIR spectroscopy is an indirect method the shape of the profile resembles the decrease in brine salt concentration during storage also shown in Figure 4.1a. Figure 4.6b shows the scores of the first PC versus the measured salt values of the brine with a Pearson correlation value r=0.96. This confirms that the change in NIR spectra can be related to the chemical changes in the brine and hence used to follow the salt changes in the herring brining process. An increasing trend in the second PC score values during processing was observed and is shown in Figure 4.7a, which resembles the increasing protein concentration measured in the brine

shown in Figure 4.5. A good correlation was observed between the second principal components and the measured protein concentration of the brine of Pearson correlation value r= 0.89 (Figure 4.7b).

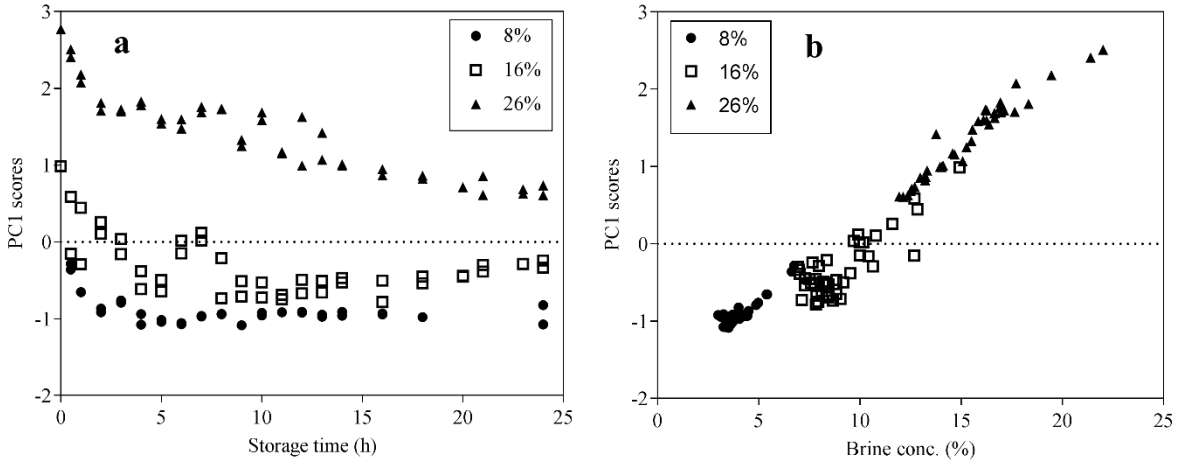

Figure 4.6: PC1 score values versus storage time from the PCA model of NIR measurements (PC1 explains 97.2 %) (a) and the PC1 scores values in function of the measured salt concentration of the brine for all three batches, Pearson r=0.89 (b) from a brine experiment ("**October 2016**") where herring fillets were brined in 8 % (●), 16 % (◘) or 26 % (▲) salt using a brine-to-fish ratio of 1:1 for 24 h (N=2 per sampling point).

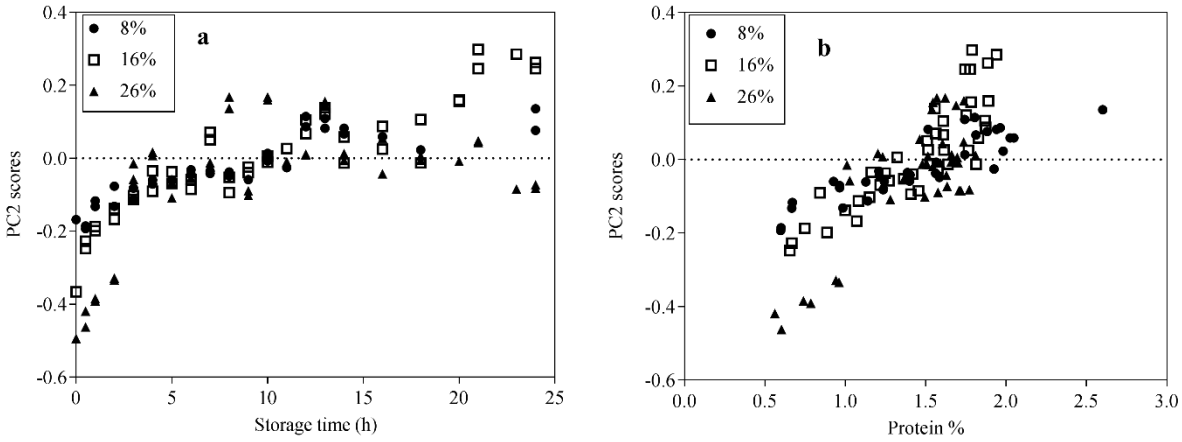

Figure 4.7: PC2 score values versus storage time from the PCA model of NIR measurements (PC2 explains 2.5 %) (a) and the PC2 scores values as a function of the measured protein concentration (%) of the brine for all three batches, Pearson r=0.85 (b) from a brine experiments where herring fillets were brined in 8 % (●), 16 % (◘) or 26 % (▲) salt using a brine-to-fish ratio of 1:1 for 24 h (N=2 per sampling point).

These results could potentially be used to determine when the system is in equilibrium i.e. and possibly detect differences in process conditions, variation of the raw material and variation in batch profiles (Lyndgaard, Engelsen, & Van Den Berg, 2012). Moreover, the linear relationship between the NIR spectra and the salt and protein concentration in the brine indicates that NIR spectroscopy potentially could be used as a fast method to predict the salt and protein content simultaneously. PLS models were conducted for both salt and protein and proved promising (results not shown).

The NIR spectra were obtained on the brine samples that were centrifuged prior to measurement in order to remove larger tissue particles that could scatter the light. The sample pre-treatment as well as mathematical pre-processing of the NIR spectra were conducted in order to enhance the chemical information related to the change in salt concentration during processing. However, the change in the brine composition during processing due to simultaneous counter diffusion of water, salt, soluble proteins, small peptides etc. also affect the NIR spectra and may give further information of the process dynamics occurring.

Regularly in the industry, the intermediate brining process often results in un-even distribution of salt in the herring fillets because of variation in the brining time as well as the stirring time while brining. The use of NIRS to monitor the process could potentially lead to a more automatic production system, where the change in salt concentration, could be monitored in real-time. To monitor real-time change in salt concentration on-line NIR measurements in-situ could be beneficial because of the chemical and/or physical information for the following process dynamic. However, much emphasis should go into the choice of sampling position as the concentration of salt may be different at different places in the tank or vat. The brine composition changes during processing with an increase in fish particles and soluble proteins etc., which can lead to changes in the NIR measurements and if stirring is used then it should be kept constant as changes in stirring may change the absorbance of light because of faster movement of fish particles and air bubbles (Tamburini, Vaccari, Tosi, & Trilli, 2003).

In our results, we have investigated the changes in PCA score values over time, which could be related to the change in salt concentration of the brine (PC1 scores) and the increase in protein (PC2 scores) over time. This indicates that the score values can be used in itself to determine when the brine concentration reaches a steady state, however, further exploration of the process dynamics could also be done by fitting suitable kinetic models to the time profiles of the PC scores (Svendsen, Skov, & van den Berg, 2015). The kinetic model parameters can then be extracted and used as input variables for prediction of the process time in real-time (Lyndgaard et al., 2012). This is illustrated in Figure 4.8, where we have fitted the model described in Paper

I using equation 4 to the time profiles of the first PC from a PCA model of the NIR measurements on brine from a herring brining process using 8 % salt. The diffusion coefficient determined on the basis of the score profile of 1.61 x 10^{-9} m^2/s over time is similar to the salt diffusion coefficient (2.31 x 10^{-9} m^2/s) obtained on the basis of the global concentration profiles in Figure 4.1a. This illustrates that fast NIR measurements of the brine can be used to describe the decrease in salt concentration of the brine as an uptake of salt in herring occurs because of a low brine-to-fish ratio (1:1).

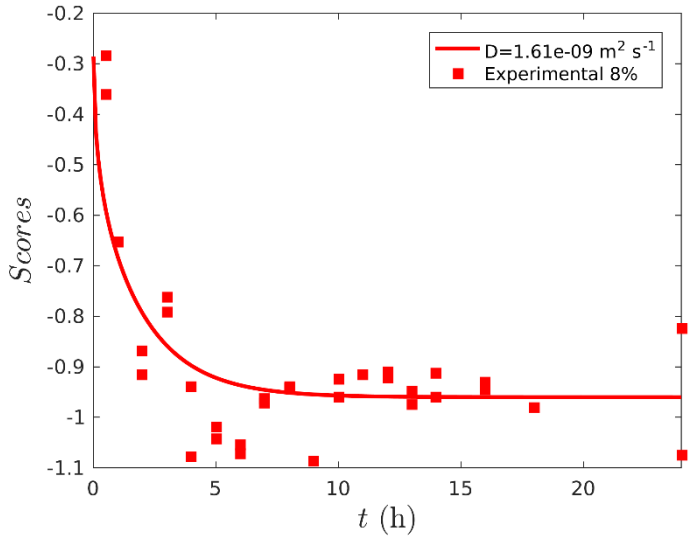

Figure 4.8: PC1 score values versus storage time from the PCA model for 8 % brine, where herring fillets were brined for 24 h. Where the discrete points represent the experimental data, while the solid line represent the predictions of equation 4 from Paper I. PC1 explains 97.2 % of the variation in data.

This allows for faster process control compared to off-line chemical measurement of the salt and other parameters. NIR measurements of the brine during the process could also be used for PLS prediction to determine the development in salt/protein concentration of the brine. PLS prediction of the salt concentration can be combined with statistical process control (SPC) to describe process abnormalities and deviations from the target values of e.g. salt.

Summary of the brining process

In summary, this section presents the results concerning salting of herring fillets, which is commonly used as an intermediate step in the herring marinating process. These studies include investigations of the salt and water uptake of herring fillets during 24 h of brining using the 1:1 brine-to-fish ratio, which consequently leads to a decrease in the brine concentration and a weight gain in the herring fillets. A simple model was developed for the prediction of the salt concentration during the brining process, which was based on the development of salt concentration over time hence global concentration profiles. Additionally, a more complex model was developed to describe the coupled transfer of salt and moisture, which was based on local concentration profiles showing the distribution of salt and water inside the fillets and the time induced changes on the profile shapes.

NIR spectroscopy was used to study the process dynamics in brining of herring fillets. The change in brine composition during storage was mainly observed by a decrease in salt concentration and an increase in protein concentration in the surrounding brine. A PCA model was developed of the NIR measurements of the brine and the time profiles of the PC scores resembled the change in salt and protein, which indicated that NIR has the potential to be used as a method for continuous process control.

4.4 Brining and marinating

During this project, numerous herring marinating experiments were conducted, which consisted of a preliminary salt brining step followed by a marinating step using salt and acetic acid. Our studies were based on laboratory scale experiments, however, simulating the industrial process. The overall aim with the herring marinating experiments, was to investigate and explain the underlying mechanisms for the varying weight yield (outcome) of marinated herring products, with focus on the effects of the salt brining process. In order to uncover the relation between process parameters, product yield and the raw material properties, it was necessary to conduct studies at the level of individual herring fillets, which makes this study unique. The fat content was measured on each single fillet for these storage experiments and a great variation in the fat content was found between the batches as described later in section 4.7. The fat content affected the weight change and overshadowed the effect of the process conditions. For that reason, it was necessary to divide the studies according to the variation in fat content of the herring fillets in order to investigate the effect of the processing conditions.

4.5 Fillet weight change

In general, the herring fillets increase in weight during the brining process due to uptake of salt and water as a consequence of the brine-to-fish ratio (1:1), which was discussed in section 4.1. However, in the following marinating procedure, where the herring fillets were immersed in a solution of acetic acid and salt, the fillet weight decreases during storage (Figure 4.9). The main weight change generally occurred within the first few days of marinating. This is seen as the steep decrease in the curves in the beginning of the process shown in Figure 4.9a and b. However, the final weight yield depended on the acetic acid concentration in the marinade, and greater weight loss were seen for the fillets marinated in 6 % and 9 % acetic acid, which is shown in Figure 4.9a.

The recommended storage time in the manufacturing of pickled herring products is 5-17 weeks depending on the concentration of salt and acid (EFSA Panel on Biological Hazards, 2010; Huss et al., 2004), however, often the storage time exceeds the recommended time because the consumption of marinated herring products changes and increases during Easter and Christmas time. To cope with the change in demand, the herring manufactures often intensify the production many days before to meet the higher demand (Szymczak & Kołakowski, 2012). Our results show that only minor changes in the herring fillet weight occurred when fillets were stored up to 100 days (Figure 4.9b (●)) as well as for 365 days (results not shown). The

components transferred in the beginning of the marinating process is mainly water and salt, which accounts for most of the weight change in herring fillets. In contrast, the smaller weight decrease later in the process could be due to diffusion of soluble proteins, peptides, other nitrogen fractions and fat (Szymczak & Kołakowski, 2012).

The storage time for the intermediate brining process was studied in relation to the effect on the weight change observed for the herring fillets during the following marinating process. We found no great difference in the weight change for the herring fillets during the marinating process if they were brined for 24 h, 48 h or 4 days. This might be explained by the relatively fast equalization of salt concentration in the fish and brine found in the brining experiments described in section 4.1. However, a continuous weight gain in herring fillets were observed during storage up to 7 days under similar conditions (Birkeland et al., 2005). For the herring fillets that were not brined prior to marinating a faster decrease in weight was observed and they ended with the lowest final weight yield observed shown in Figure 4.9b (▲). Paper I provides further details of the experimental results and the difference between the experimental batches.

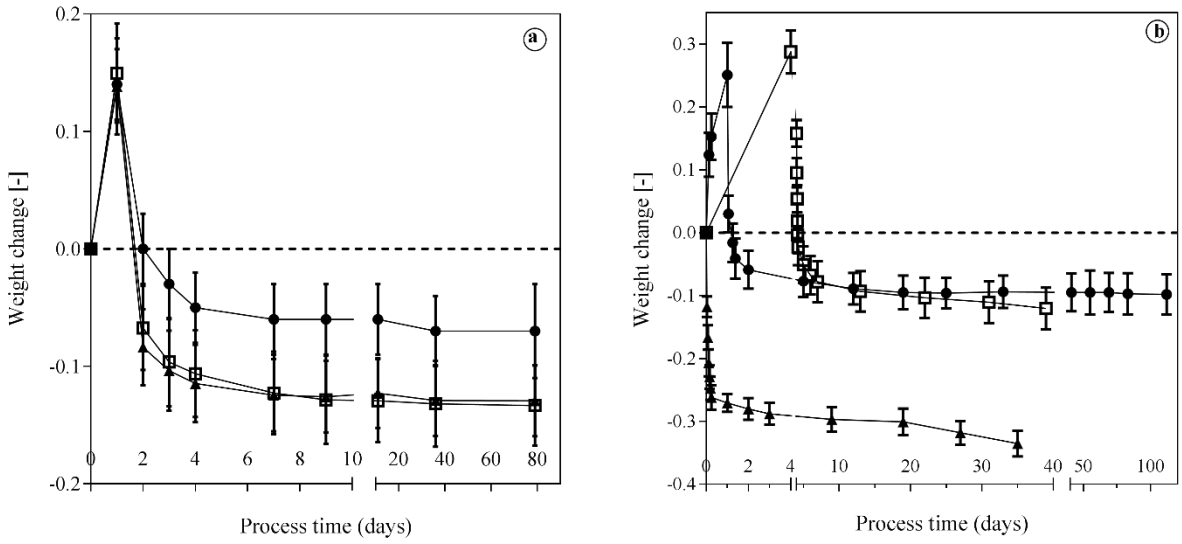

Figure 4.9: Change in fillet weight during brining (13.6 % NaCl for 24 h) and marinating in 4.3 % NaCl with 3 % (batch B) (●), 6 % (batch C) (□) and 9 % (batch D) (▲) acetic acid (a). Weight change in herring fillets during brining for 24 h (batch F) (●), 4 days (batch H) (□) or no brining process (batch I) (▲) (b) and marinating in 5.4 % NaCl and 5.8 % acetic acid (Batches are further described in Paper I).

Even a small increase in product yield in an industrial production has a great economic effect. Adjusting the acetic acid and salt concentrations in the herring marinating procedure without compromising food safety could possibly lead to a change in product yield hence a change in the weight reduction during storage. Herring fillets were brined (in 13.6 % salt for 24 h) and

subsequently marinated in 6.5 % acetic acid and 4.3 % salt (Marinade 1) or in 5 % acetic acid and 5.8 % salt (Marinade 2) in order to compare the weight changes of individual herring fillets. The average fat content of batch 1 and Batch 2 was 3.5 % and 4.6 %, respectively, and both batches ranged within 1.2 % to 9.7 % fat. Herring fillets increased in weight during the brining process with a total increase of 25 % and 22 % for Marinade 1 and 2, respectively. In the subsequent marinating process the fillet weight was reduced, where fillets in Marinade 1 decreased faster in weight compared to fillets in Marinade 2 shown in Figure 4.10.

The fillets in marinade 1 were subjected to a higher acid concentration, which lowers the pH and causes a drop in the water holding capacity (Rodger et al., 1984; Szymczak et al., 2012), which can explain the higher weight loss observed for this group of herring fillets.

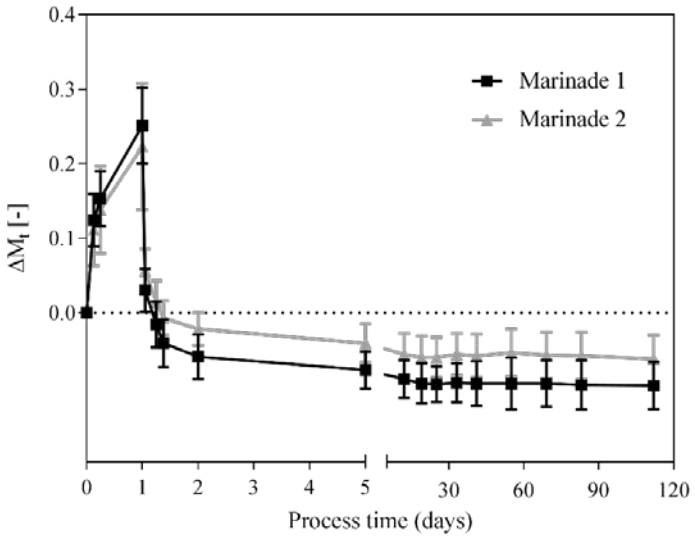

Figure 4.10: Fresh herring fillets from April 2015 were brined in 13.6 % NaCl for 24 hours and subsequently marinated in 6.5 % acetic acid and 4.3 % salt (Marinade 1, N=19) or in 5 % acetic acid and 5.8 % salt (Marinade 2, N=17).

The results of the two experiments were further confirmed in a pilot study conducted in an industrial set-up, which showed the same difference in final product yield at the end of the marinating process. The similar results obtained for the pilot study and the laboratory experiments indicate that the conducted laboratory experiments during this project can be thought of as a simulation of the industrial process conditions at least for pilot studies.

4.6 Raw material variation

Studies of fish marinating have mainly been conducted batch wise (Birkeland et al., 2005; Szymczak & Kołakowski, 2012; Topuz, 2016), where it is often assumed that batches are homogenous groups, even though it is well known that variation within batches does occur. It is well known that the fat content, which is often regarded as an important quality parameter for herring products, varies substantially in herring fillets. Investigating the herring marinating process at batch level without taking the variation within the batch into account results in loss of valuable information concerning the effect of the biological variation in fat content on the process yield. A large variation in the fat content was found between batches of herring as well as within one batch, which is showed in Figure 4.11. The highest fat content was observed for the months of June and December, where the lowest fat content was observed in April.

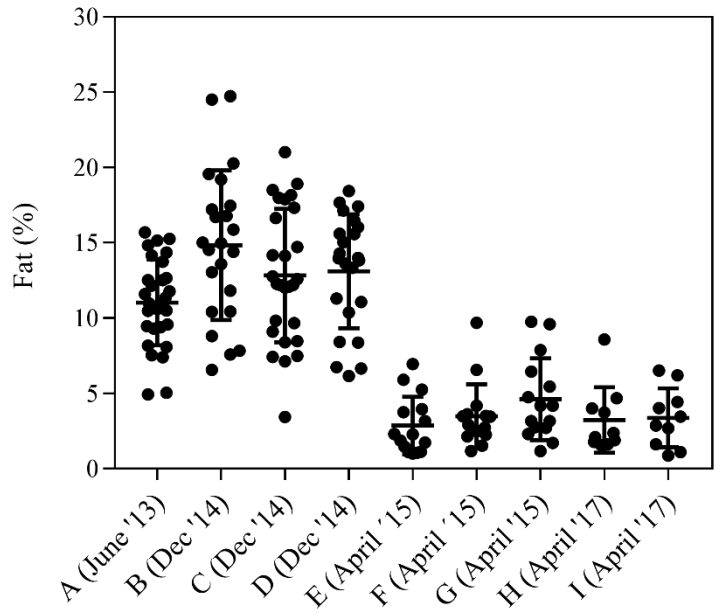

Figure 4.11: The fat content (%) in raw herring fillets included in the marinating experiments presented in paper I.

We compared the fat content of the raw herring fillet (one part of the butterfly fillet) with the total weight change of the marinated fillet (the second part of the butterfly fillet) and found a good correlation between the two, showing that with the increasing fat content in herring fillets, the weight loss that occurred during processing decreased (further details are given in paper I). This implies that fatty herrings had less water to lose during the marinating process, which would be beneficial for the manufacturers in achieving a higher product yield. We suggest that

the correlation between the weight change and the total fat is due to the fat acting as a passive component in the transfer mechanisms. And it is the amount of water phase present in the muscle that determine how much water there is available to diffusional exchange with the surrounding marinade.

4.7 Texture changes

A major part of the project has aimed at evaluating the changes in process conditions on the product yield of marinated herring. However, the changes concerning product quality such as texture are equally important from a consumer's point of view. The fish muscle structure is affected by the brining and marinating process, and have it this project been investigated by the use of TPA and simple compression test measuring hardness.

4.7.1 Texture profile analysis

The parameter "Springiness" was chosen as the parameter to study the textural changes in the herring fillets during processing. Springiness is not affected by the sample height, which was an advantage as the herring fillets varied in thickness because of the natural variation. The changes in springiness in function of process time are shown for two representative batches of herring fillets in Figure 4.12a. Springiness value decreased during the marinating process, where the greatest change was seen in the beginning of the process. Lower springiness values were obtained for herring fillets that were not brined prior to the subsequent marinating process (batch I) compared to the fillets that were brined for 4 days (batch H) prior the marinating process. These results correspond to the change in weight during the process, where the fillets with no prior brining (batch I) decreased faster in weight compared to the brined fillets (batch H), which was presented in section 4.5 and Figure 4.9b. A Pearson correlation of r= 0.71 was found between the springiness and the weight change for marinated herring fillets shown in Figure 4.12b. During the brining process the fillet weight increased due to uptake of salt and water, which resulted in a high springiness value.

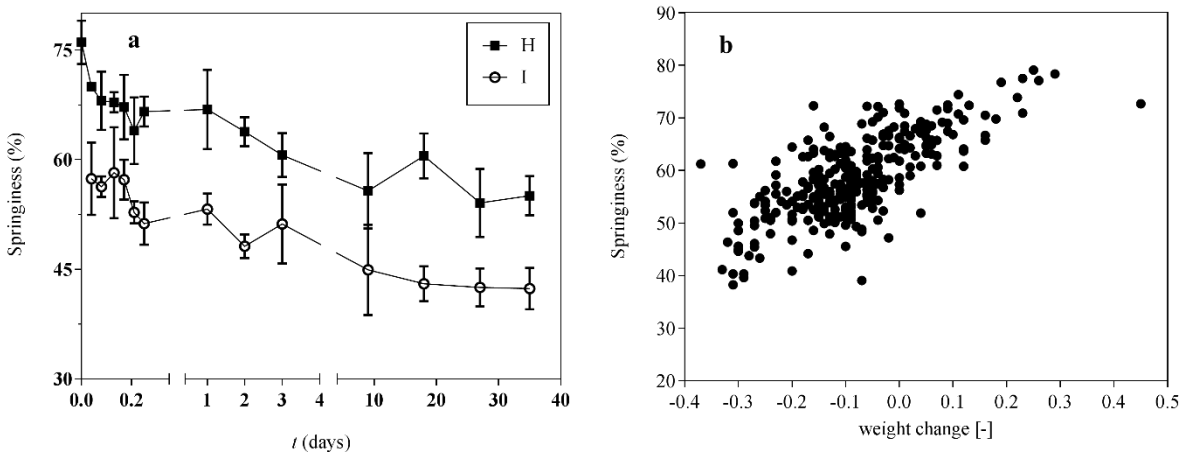

Figure 4.12: Change in springiness during the herring marinating process for batch H (■) and batch I (○). Batch H was brined for four days and batch I was not brined prior marinating (a). Springiness versus the weight change for seven batches of marinated herring fillets, N=289, Pearson r =0.72 (b). Every batch was marinated in 5.4 % NaCl and 5.8 % acetic acid using a brine-to-fish ratio of 1:1.

The sample size has a great effect on the TPA measurement and the parameter hardness is greatly affected by this. As the herring fillets vary in thickness and this was not adjusted for during measurements, only the springiness was evaluated as it was not related to the hardness. It was chosen not to uniformize the sample thickness in order to not affect the muscle structure too much and hence affect the texture measurement. Even though more texture parameters can be determined using TPA, evaluation of hardness using a single compression with a spherical probe on the whole fillet is less destructive and may be easier to conduct in an industrial set-up. For industrial use, the methods needs to be easy to use with good reproducibility in the daily quality control.

4.7.2 Evaluation of hardness

Fresh herring fillets have mainly been studied in this work, but commonly the industry also use frozen material due to changes in demand and raw material availability. For that reason, the weight change as well as the change in hardness during the marinating procedure was investigated in pre-frozen herring fillets and compared to fresh fillets. There was no significant difference in the weight gain during intermediate brining process between fresh and defrosted fillets. However, during the marinating process, the fillets that were previously frozen decreased in weight more quickly than the fresh fillets, with a significant difference between the groups from day 4 and onward ($P<0.05$) (Figure 4.13). The lower obtained yield for the pre-frozen fillets compared to fresh fillets during the marinating process, can be explained by the lower water

holding capacity due to the change in protein conformations and protein/water interaction during freezing.

It is known that the textural properties in fish muscle changes during frozen storage and the herring fillets are subjected to salt and acetic acid. Texture analysis was conducted on whole herring fillets by measuring the hardness at four positions on each of the fillets from the two groups: frozen and fresh herring (N=3). Three fillets from each groups were selected for texture evaluation at each time point in order to follow the change in hardness during brining and marinating. The first sampling point of the fillets (position 1) was nearest the head region and the fourth sampling points (position 4) was closest to the tail region. The results for the evaluation of hardness during the salting and marinating process of the herring fillets are shown in Figure 4.14 a-g, where each figure represents each sampling day during the process.

In general, higher hardness values were seen for the defrosted fillets compared to the fresh fillets, which corresponds to the higher weight change observed for the defrosted fillets that were shown in Figure 4.13. The fillets that were frozen showed higher values for hardness compared to the fresh fillets prior to the salting process, which is shown in Figure 4.14a. This can be due to the alteration of the muscle structure during frozen storage leading to a toughening of the muscle (Sjofn Sigurgisladottir, Ingvarsdottir, Torrissen, & Cardinal, 2000). During brining, the hardness decrease for both groups of herring fillets (fresh and defrosted) at position 1 and 2) (Figure 4.13b), which is possibly explained by the increase in weight due to water and salt uptake shown in Figure 4.13. After brining, the fillets were immersed in the marinade causing an increase in the hardness values at all four positions for both groups of herring fillets, which is seen by comparing Figure 4.14 c to d. During the marinating procedure, the acetic acid diffuses into the herring muscle, which lowers the pH causing protein denaturation and lower water absorption (Szymczak, 2011) and an increased firmness (Rodger et al., 1984). This can explain the weight decrease observed for both groups of fillets shown in Figure 4.13 and the increase in the maximum force from day 1 to day 2 (figure 4.14c-d). No greater changes occurs from day 3 to day 6.

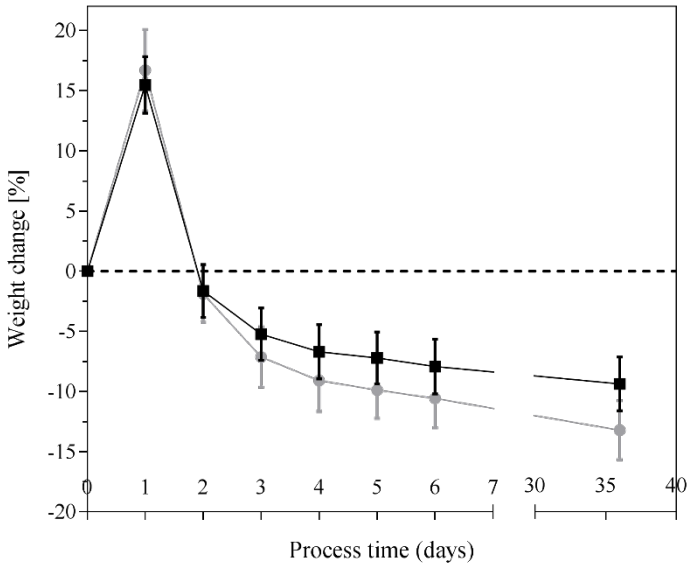

Figure 4.13: Change in fillet weight (%) during brining (13.6 % NaCl) and marinating (5 % NaCl and 6.6 % acetic acid) of fresh (■) and frozen herring (●). Herrings were caught around July 2014 and frozen for app. 2 months at -40 °C. Fresh herrings were caught in end August 2014 (Further details are given in Paper I).

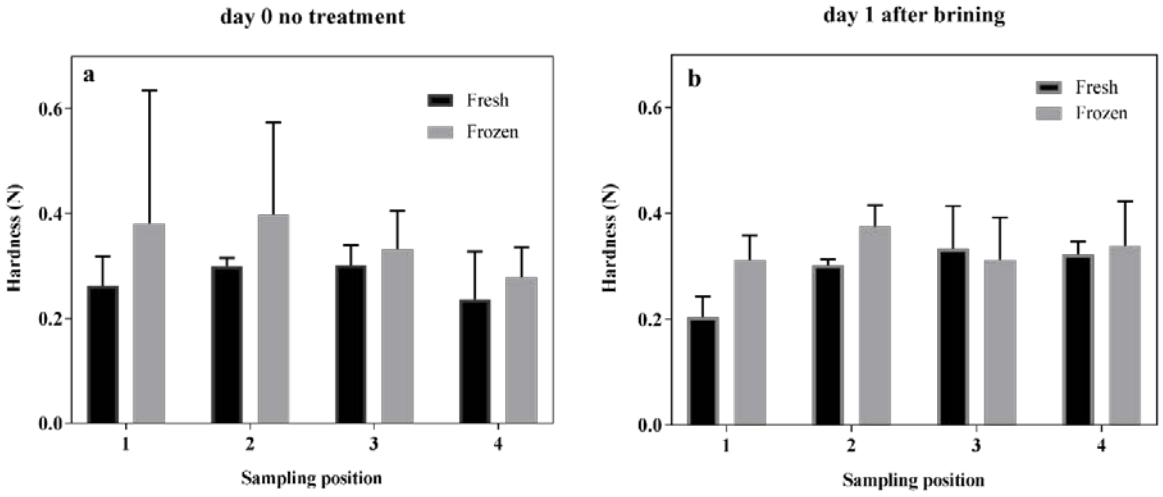

Figure 4.14: Hardness of herring fillets measured by a 10% compression using a cylindrical probe at four locations from the head to the tail. Data are mean and standard deviation of 3 fish each time point, day 0= no treatment (a), day 1= after 24 h brining (b) and day=2-6 (c-g) are the marinating process. Herring fillets were brined for 24 h (13.6 % NaCl) and subsequently marinated (6.5 % acetic acid and 5 % NaCl).

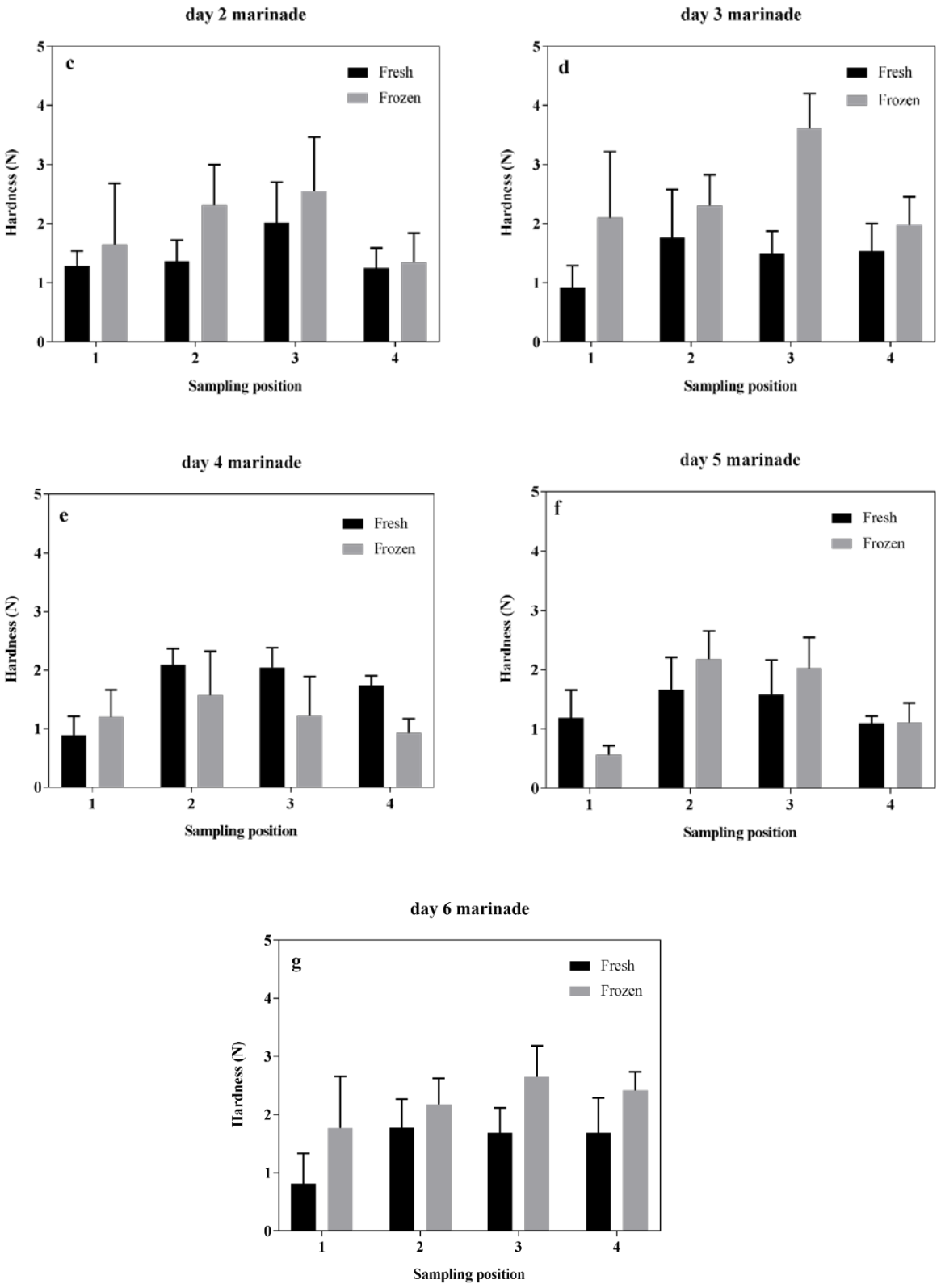

Figure 4.14 continued

For both groups of herring fillets, the maximum force at position 1 and 4 seems to be lower than at position 3 and 4, which emphasizes the importance of the sampling position and the presentation of the results. In general, a large standard deviation is seen for the measurements conducted on the frozen herring fillets, which can be due to the fact that the fillets curled during processing, and were very difficult to perform the texture analysis on. The herring fillets varied not only in thickness but also in length and it was aimed to distribute the hardness measurements evenly along the fillet, however, differences in sampling position from fillet to fillet occur which partly might explain the standard deviation within each sampling position. Choice of probe size is relevant considering the possibility of fillet gaping, if measurements are conducted using a small sphere for example, the probe will go through the muscle surface and lower maximum force values are obtained than expected. Compression tests are sensitive to parameters such as sampling location, settings (e.g. degree of compression in % or with a constant penetration depth), type of probe (Hyldig & Nielsen, 2001) and sample temperature (Nielsen et al., 2005b). The reproducibility of the texture measurements may depend on the sampling location because of the possible heterogeneity of the fillets and finding a representative sampling location on the fillet is important.

4.8 Measuring of salt using NIR Spectroscopy

Ensuring an adequate salt concentration in the marinated herring fillets is one of the most important safety parameters in the manufacturing process of marinated herring products (EFSA Panel on Biological Hazards, 2010; Huss; Ababouch; Gram et al., 2004). Controlling the salt concentration in the fillets are often done at the end of the process by chemical methods, which is a destructive and time-consuming task. NIRS was explored as an alternative method to determine the salt content in a non-destructive and fast manner with the idea of sampling the surrounding brine/marinade instead of individual fillets. This was considered to be more representative for the entire batch of herring fillets as they are known to vary in raw material composition. However, a prerequisite for indirectly determining the salt concentration in the fish by obtaining NIR spectra of the marinade is that the salt concentration should be in equilibrium between the fish muscle and the surrounding medium.

Results from our previous studies using NIR in the brining of herring fillets presented in section 4.3 indicated that NIR spectroscopy could work quite satisfactory to determine the salt concentration in brine even though NaCl has no specific absorbance bands in the NIR spectra. Figure 4.15a and b shows the two PLS models that is described in Paper III consisting of the data sets “April 2017” (described in section 3.3); the first model predicting the salt concentration

in the marinade with the prediction error 0.27 % (RMSECV) and the second model predicting the salt concentration in the fish water phase with the prediction error 0.41 % (RMSECV). The details of the prediction errors obtained are also shown in Table 4.1. The two PLS models are based on data where equilibrium of salt has been reached. It was possible to predict the salt content in the marinade at any point during the marinating process; however, the change in the NIR spectra did not explain the concentration changes in the fish muscle when the system was not in equilibrium. Figure 4.16 demonstrates the prediction of salt in the fish water phase including samples not in equilibrium also shown in table 1 in Paper III, where further details of the development of the models and experimental set-up are given.

The study was based on herring marinating experiments executed in April 2017, where the intermediate brining process varied in relation to storage time and salt concentration and the following marinating process was similar for all batches. As the study was based on experiments conducted within the same month, April 2017, no information of the seasonal change in herring was included in the model. Considering the large variation in the fat content that is observed between batches of herring but also within batches (Nielsen et al., 2005a) this may reasonably affect the composition of the marinade hence the NIR spectra. Given the heterogeneous nature of the raw material it is suggested that more realistic results could be obtained by including samples from different seasons in order to achieve a more robust and global model.

The choice of spectral region where the salt concentration changes are studied was in our study based on inspection of linear effects in NIR spectra as a consequence of salt concentration changes of pure salt solutions as well as calculating the correlation coefficient for each wavelength and the determined salt value for the herring marinating experiments. However, more automatic methods for wavelength selection are available for the development of a calibration model. Triadaphillou et al., (2007) has reviewed some of the wavelength selection strategies, which they grouped into three categories: dimension-wise selection, model-wise selection and subset selection. The authors focused on the latter method, which includes interval variable selection (iPLS) and generic algoritms (Gas) that are commonly applied methods (Triadaphillou et al., 2007). iPLS is a methods that calculates local PLS models based on spectral subintervals of equal width and compares the model performance of each local model to the global model using the full spectral region. GAs are optimization methods where individual wavelengths can be selected up to the total number of wavelengths available. However, a drawback of this method is that it may generate models that are too specific to the calibration data set, and may not be able to predict the future samples very well (Triadaphillou et al., 2007). The variable selection methods in my opinion should be used in combination with a good understanding of the spectral changes during processing and changes related to the analyte(s)

of interest. However, the automated variable selection methods give a good overview of the most relevant spectral regions to be included in the calibration model of the analyte of interest.

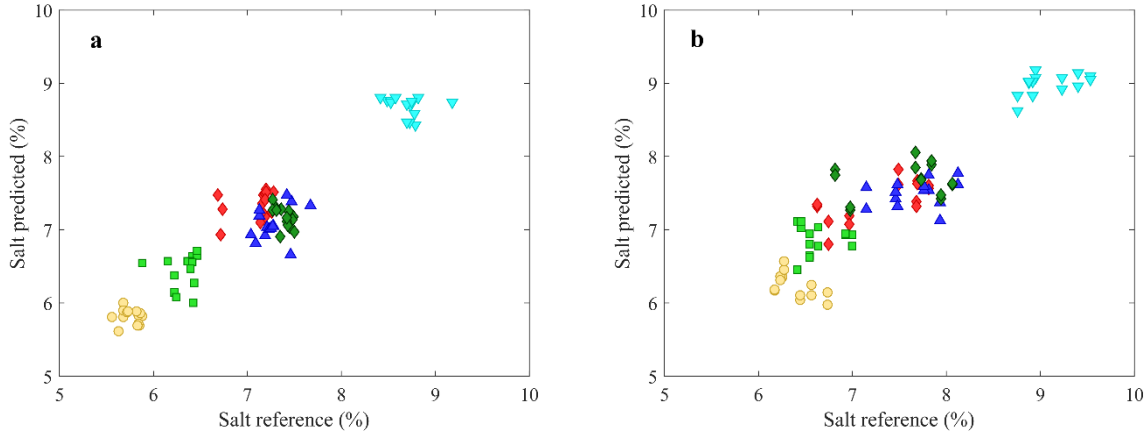

Figure 4.15: Predicted versus measured plot of salt concentration in in marinade (a) and in the fish water phase (WPS) (b) for PLS models based on selected NIR spectra region (1170-1290nm). Each color represent one batch (further details are given in Paper III). The models are developed using the data set "April2017".

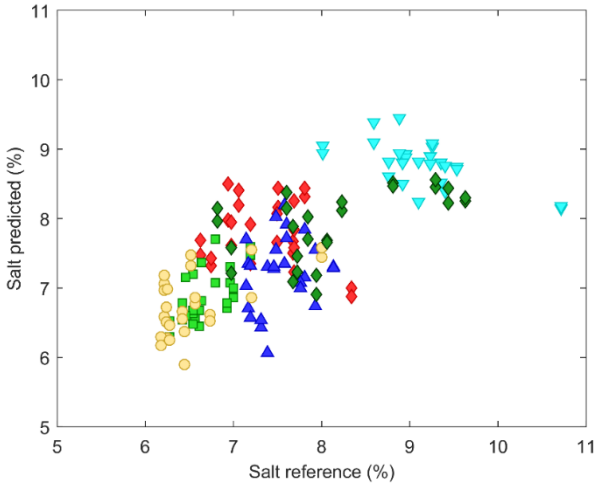

Figure 4.16: Predicted versus measured plot of salt concentration in the fish water phase for a 4 factor PLS model base on selected NIR spectral region (1170-1290 nm, R^2 =0.56, RMSEC/CV 0.63%/0.68%).

Going from lab based models to application in the industry further steps are needed. Building multivariate models for controlling industrial processes, e.g. a calibration model that is able to predict new and unknown samples, is a complex and time-consuming task, and gathering information about process and raw material variation is important. Lab models are a good first approach to study the feasibility of NIR for process control, however, building calibration models to be used in large scale industrial food processes should be conducted in the industrial

setting. The samples chosen for the calibration should span the variability both in the process (such as storage time, temperature, humidity and raw material variability) and the target constituents (such as variation in salt and acetic acid concentration).

Emphasis should be put on correct sampling to ensure that both the NIR measurements and the reference sampling (that is required for making calibration models) are conducted with a good representation of the entire batch. The principles of theory of sampling (TOS) can be applied when deciding on the sampling method in the specific production. TOS provides a description of correct sampling methods and quantification of the sampling error etc. and a standard reference to use is Gy (1998). In an industrial manufacturing process herring fillets are further processed in barrels using acetic acid and salt following the brining process. It has been shown that the salt concentration varies in the barrels and sampling conducted in the top, middle and bottom may result in different concentration values. Therefore, sampling position of brine must be kept constant in order to limit the variability in measurement. Obtaining NIR spectra of the marinade could potentially be conducted directly in the barrels using a handheld NIR device or samples could be extracted from the barrels and measured off-line. However, when measuring directly in the barrels without the possibility of sample preparation more fish particles will be present, which scatter the light hence create new demands for spectral pre-processing.

Before going all the way to the industrial process, further investigation should be conducted in the laboratory to explore the effect of the raw material variation and changes in processing conditions. The variation of the raw material is especially related to the variation in fat content as mentioned previously. Process changes could include variation of the salt and acetic acid concentrations and the different process steps; the intermediate brining and the following marinating, all of it affects the fish muscle and the composition of the brine/marinade. Control of the salt content is conducted in both steps of the marinating procedure and development of a global calibration model that can be applied to both steps would be favorable. Moreover, as introduced earlier the storage time often varies in the industrial process due to a change in customer demands and the availability of the raw material, where smaller changes in the product yield and continuous diffusion of proteins, peptides etc. from the herring fillets to the marinade occurs changing the marinade composition. The results presented in Paper III were based on experiments with a limited storage period of 35 days, which is often considered the minimum storage time in an industrial process, however, data from longer storage experiments could be interesting to investigate as well.

Further investigation of the effect of seasonal and process variation in the prediction of salt using NIRS was conducted. Data included in the investigation consisted of four experimental

sets with varying storage time, process conditions and seasonal variation of the herring fillets. Figure 4.17 illustrate the correlation between the measured salt concentration in the fish water phase (WPS) and the measured salt concentration in the surrounding salting medium for all the experiments included in the four data sets. A Pearson correlation coefficient was calculated after equilibrium of salt was reached between the salting medium and the fish muscle for the different experiments and was r=0.97.

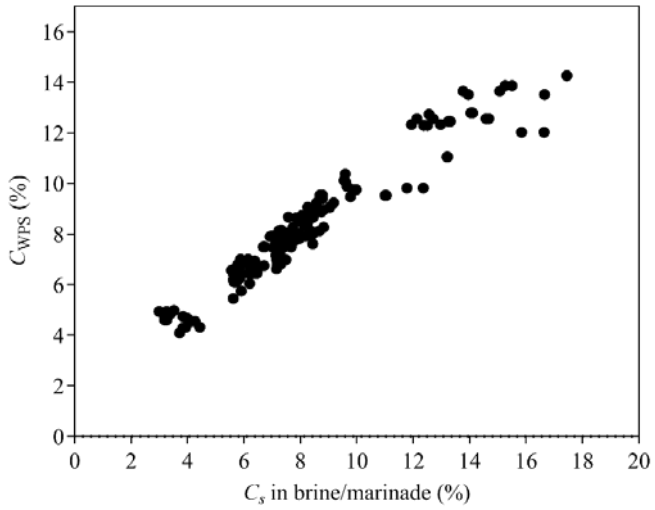

Figure 4.17: The measured salt concentration in the fish water phase (WPS) plotted against the salt concentration in the brine/marinade samples after concentration equilibrium for the four experimental data sets, Pearson r=0.97, N= 204.

PCA was applied to explore the effect of different processing conditions, storage time and seasonal changes on the NIR spectra. PCA was conducted on the spectral region 1170-1290 nm because of the high correlation between this region and the actual salt values, which is described in further details in Paper III. Figure 4.18a shows the PC2 versus PC1 scores, where samples are classified according to data set. The samples group reasonable well according to the data set they belong to except for some samples belonging to the data set “Oct2016” and “old”. This could be explained by the absence of acetic acid in those samples. Samples belonging to “Oct2016” only consisting of brine samples are more dispersed compared to the other data set, which might be explained by the fact that sampling occurred more frequently compared to the other experiments and the storage time was limited to 24 h where the greatest changes occurs in salt and water transport. Figure 4.18b illustrates the grouping of spectra according to the sample composition: brine or marinade samples.

PCA modelling was applied leaving out the data set “Oct 2016” in order to investigate the data sets containing the samples from the marinating process and to study the effect of storage time. The score plot of PC1 versus PC1 is shown in Figure 4.19a, where the samples are colored according to data set. Two distinct features were observed, one being that samples with long storage time (“old” min 3 years) having positive PC1 score values, whereas samples with shorter storage time (“Dec 2017” max 26 days) having negative PC 1 scores. The other being that each of the data sets are divided in groups depending on the concentration of salt, which is confirmed in Figure 4.19b. Figure 4.19b indicates the relation between the spectra and the salt concentration of the brine and marinade, which is mainly described by the second PC: samples with high concentration of salt having negative PC2 values, whereas samples containing lower concentration of salt having positive PC2 values.

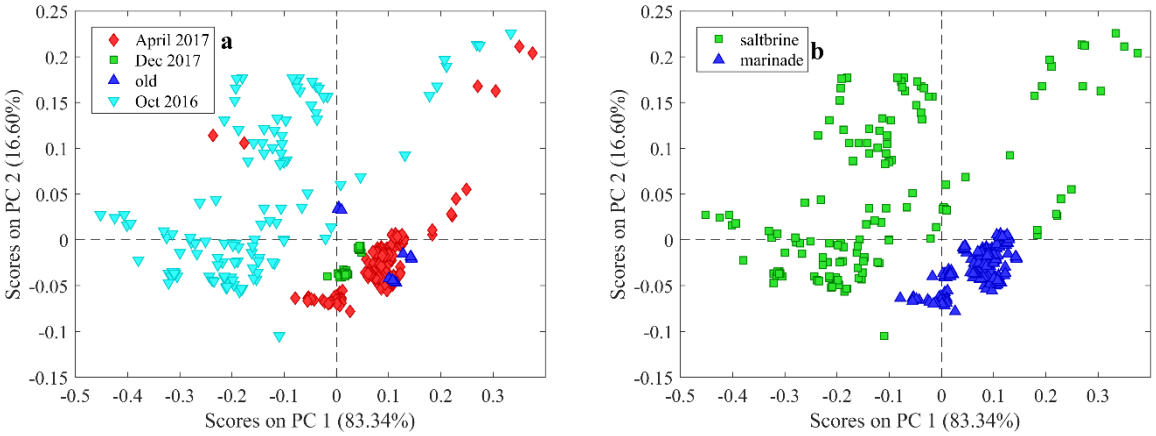

Figure 4.18: Score plot of PC2 versus PC1 from a PCA model explaining the batch variation (a) and sample type (b) for the NIR region 1170-1290 nm. Data sets are presented in section 3.3.

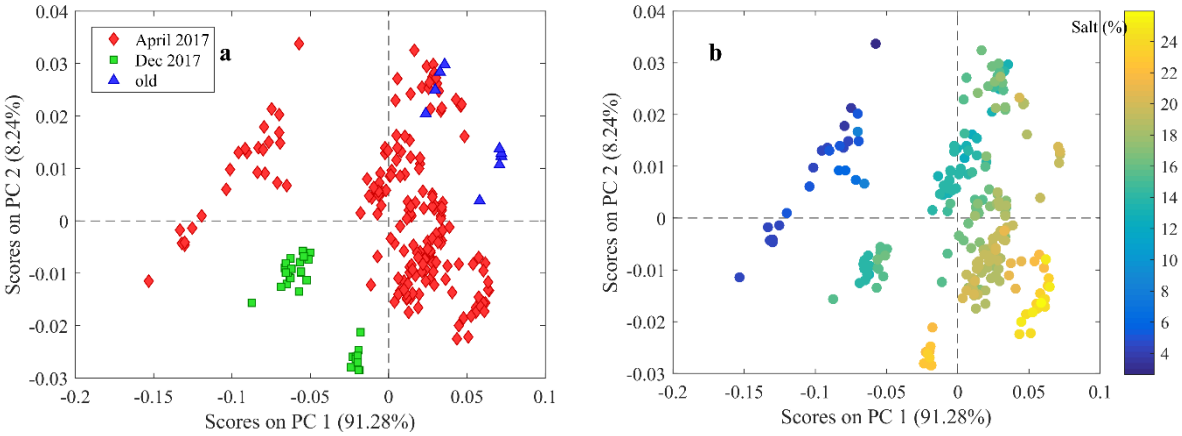

Figure 4.19: Score plot of PC2 versus PC1 from a PCA model explaining the batch variation (a) and the salt concentration (b) for the NIR region 1170-1290 nm.

PLS regression was applied on the four data sets with the aim at building a global prediction model applicable to the combined brining and marinating process for predicting salt content in the surrounding brine/marinade. The wavelength region, 1170-1290 nm, was included in the calibration model based on the previous investigations also described in Paper III. For the sake of comparison, two models were built; one using all samples from the data sets (Figure 4.20a) and the other using samples after equilibrium of salt was reached between the fish muscle and the brine/marinade (Figure 4.20b). The model validation error (RMSECV) was similar for the two PLS models 0.53 % and 0.52 %, respectively. More factors were included in these two models, 7 LV, compared to the model obtained in Paper III (5 LV), which can be explained by the fact that more factors are needed in order to explain the greater variation in process conditions as well as seasonal variation of the raw material. Further details of the model validation errors are given in Table 4.1. A final PLS model was built to determine the salt content in the fish muscle from NIR spectra of the brine/marinade resulting in a 7 factor model and a prediction error of 0.72 % (Figure 3.21).

The number of samples included in the PLS model for the water phase salt (Global) shown in Figure 4.21 was lower compared to the number of samples included in the PLS model of the salt concentration in the brine/marinade (Figure 4.20b) even though both cases included samples in equilibrium. The concentration of salt in the fish samples were not in equilibrium with the concentration in the marinade probably because of an experimental error, and were removed from the data set prior modelling.

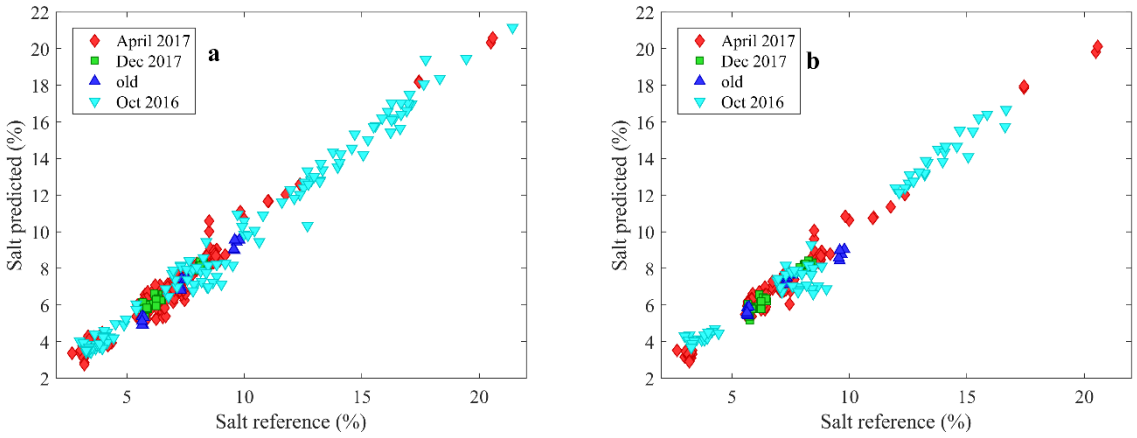

Figure 4.20: Predicted versus measured plot of salt concentration in brine/marinade (N=354, R^2= 0.98, RMSEC/CV 0.48 %/0.53 %) (a) and predicted versus measured plot of the salt concentration in brine/marinade after equilibrium (N=220) (b) for 7 factor PLS models based on selected NIR spectral region.

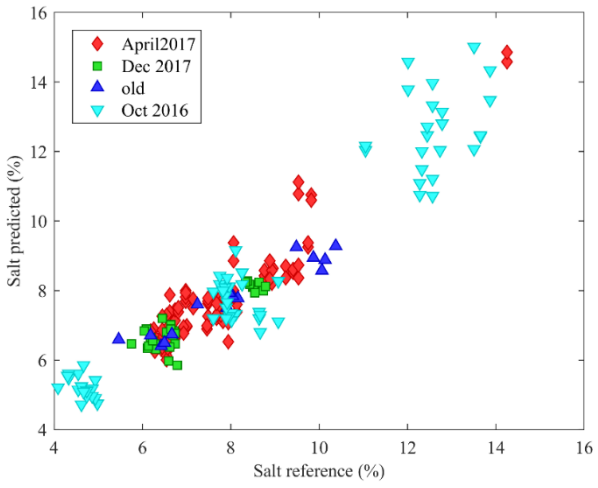

Figure 4.21: Predicted versus measured plot of salt concentration in the fish water phase after equilibrium of salt for a 7 factor PLS model based on selected spectral region (1170-1290 nm), N=204, R^2= 0.89, RMSEC/CV 0.59 %/0.72 %.

Table 4.1: Model error of validation for global and local models predicting salt content in the marinade and the fish (WPS).

PLS model	RMSEC (%)	RMSECV (%)	RMSEP (%)	LV	R^2
		Global			
Salt in marinade (%)	0.46	0.52	0.56	7	0.97
WPS (%)	0.59	0.72	0.39	7	0.89
		Local			
Salt in marinade (%)	0.26	0.27	-	2	0.91
WPS (%)	0.36	0.41	-	5	0.81

WPS: water phase salt (%), RMSEC: root mean square error of calibration; RMSECV: root mean square error of cross-validation; RMSEP: root mean square error of prediction, LV: latent variables, R^2: correlation coefficient.

External test set validation of the global model for salt in the marinade gave a prediction error of 0.56 % and for salt in the fish (WPS) 0.39 % (Table 4.1). Usually, the test set prediction error is higher than the error of calibration, but in our case it turned out to be lower for the prediction of WPS. This could mean that the model was an adequate representation of the marinating process for this batch (Tamburini et al., 2003). External validation using the "true" independent test set was also applied for the local model (Paper III), however, the model did not predict the new samples well (results not shown). This is probably because the local model does not contain information of the varying seasons, as the fish used in the test set were caught in December and stored until February, and the herrings used in the local models were caught in April and great differences in the fat content could be present. The sample size of the test-set is limited to 15 samples, which might seem a little low considering the samples size of the calibration set. An optimal test-set should include samples from varying seasons, different process conditions and so on (similar to the calibration samples). Lower validation error was obtained with the local prediction model (Table 4.1) compared to the global model, however, a limited number of calibration samples were used in making the local model and limited variation in raw material as well as process variation were introduced, and the local model may only apply for the samples in question and not for new samples from another season.

The feasibility study using NIRS in the herring marinating process was limited to study the prediction of salt in the marinade or the fish using the spectral region 1170-1290 nm. The selected spectral region was found most applicable for studying the evolution of the spectra related to changes in salt concentration, which was studied in Paper III. However, it could be

interesting to further study the relation between the physical changes in the marinade during storage and the chemical changes in the fish, where other parts of the NIR spectra could be relevant and interesting to study.

In this study we have shown the feasibility of NIR as a fast measuring method for determining the salt concentration in the marinade and fish samples in herring marinating experiments under controlled laboratory experiments. Taking the model to the next step and applying it in a production set-up require further investigations. From this point the model is based on laboratory experiments, and even though we have shown that the marinating experiments conducted for this project have similar results when reproduced in a pilot set-up in an industrial scale (section 4.5), we need to test the use of NIR in an industrial set-up. NIR measurement should be conducted in an industrial set-up and the sampling positions should be tested in order to find the most representative sampling of the entire batch of herring fillets. Moreover, the type of measurements considering conducting off-line measurements or directly measuring in the barrels. Gathering of calibration samples that span the variation of the raw material, the process conditions, and temperature variations should be prioritized in order to achieve more robust models and reliable predictions. Maintaining of the models is another important issue that should be considered and done continuously.

Summary of the brining and marinating process

Weight and texture changes in individual herring fillet were investigated during the marinating process with focus on the intermediate brining step. Our studies confirmed the existence of a great variation in the fat content in herrings both between batches and within batch. We studied the weight change in herring fillets and found that the main change generally occurred within the first few days of marinating independent of the preliminary brining process and the concentration of salt and acetic acid in the following marinating process. The fillet weight during marinating decreased with increasing acetic acid concentration in the marinade. It was found that with increasing fat content a lower weight loss was observed for the fillets during the marinating process.

Fresh herring fillets were mainly used for the experiments in this project, however, a study comparing the change in weight and texture for fresh and frozen raw material was conducted. The results showed that higher weight losses and increased hardness was observed for herring fillets that were frozen prior to the marinating process. Furthermore, it was found that the sampling position on the herring fillet for texture analysis is important to consider in order to

be able to compare the measurements. However, the natural variation in size of the herring fillets may introduce further difficulties in comparing the texture measurements between the fillets.

NIR spectroscopy was studied as a potential method for controlling the salt content in herring fillets indirectly by obtaining spectra of the brine/marinade. Reasonable good PLS models were developed for with the prerequisite that the herring/brine system was in equilibrium. Given that by including samples from different seasons more robust and global models could be obtained. NIR can be used to simultaneously determine other quality parameters, which makes it a suitable method for product and process development.

Chapter 5: **Conclusion**

The main objectives of this PhD work was to study and enhance the knowledge of the herring marinating process including the intermediate brining step in order to aid in industrial process optimization.

By conducting experiments on individual herring fillets we have gained a deeper knowledge about the effect of fat content and the process variables on the variation in product yield. These studies can be used to optimize the current industrial process. Our results from the controlled lab experiments were later repeated in pilot studies in an industrial set-up and used for optimizing current process conditions in full scale.

Studies of the herring salting process under different conditions led to development of two models: i) one model that can be used to predict the average salt concentration in the herring fillet during brining and ii) one model that predicts the coupled salt and water concentration profiles in herring fillets. These models are useful to study and enhance knowledge about salt and moisture transfer in herring when developing new products or optimizing processing conditions and storage time for industrial purposes.

We studied the use of NIRS with the aim of achieving a fast and easy control of the salt concentration in the herring marinating process. Prediction models were developed, which could be used to determine the salt concentration in the marinade, but also in the fish under the prerequisite that the system (brine/fish) was in equilibrium. NIR is a fast and non-destructive method to determine the salt content in fish using the surrounding brine/marinade and can replace the more time consuming chemical measurements of salt often used in the quality control in industry. Additionally, NIR measurements opens up for new opportunities of several parameters simultaneously.

These studies provide the basis for, and hopefully encourage, further studies of the herring marinating process and future development and application of the models in an industrial set-up to optimize the process.

Chapter 6: **Outlook**

In the present PhD thesis, three approaches are used to investigate the herring marinating process and an outlook is presented for each of these approaches.

Herring marinating experiments were conducted using a marinade-to-fish ratio of 1:1. This ratio was chosen because of its similarity to the industry standard. However, equilibrium of salt and acetic acid between the fish muscle and the marinade was reached within the first couple of days and consequently changed the concentration of the marinade as well as the concentration of salt and acid in the fish because of the low marinade-to-fish ratio. Playing around with the ratio to investigate the equilibrium concentrations in order to achieve a better control of the equilibrium concentrations could be interesting in the future; however, this may lead to other unwanted factors may be introduced such as higher protein extraction from the muscle and consequently a quality degradation.

In this work, it was investigated how the acetic acid concentration of the marinade affected the weight change during processing. It would be necessary to investigate the effect of different salt concentrations and the effect on product yield during long-term storage to get a better understanding of the effect of different processing conditions,

A model was developed to describe the salt and moisture transfer during salting of herring fillets. To further improve this it is desired to develop a model that describes the combined process of salting and marinating including the diffusion coefficients of acetic acid. Therefore, further experiments of the acetic acid, salt and moisture profiles are necessary and determination of the diffusion coefficient of acetic acid is needed in order to fulfill this.

As is confirmed by our studies, herrings vary greatly in fat content between and within batches. We need to investigate if the varying fat content in herring affects the model prediction of the concentration profiles as well as the use of different salting and marinating conditions. Adding this knowledge about the effect of fat on the diffusion of salt and acetic acid in the model could be useful for industrial purposes in order to optimize the marinating procedure with seasonal changes of the raw material.

The concentration profiles were investigated by measuring the concentration of the solutes inside the fillet, however, to further visualize the effect of salt, it could be interesting to study

the microstructural changes using microscopic methods and the aspects of acetic acid as well. The model predicting local concentration profiles was developed based on chemical measurements of the samples using a manual cutting method. In order to optimize the model, it would be necessary to improve this cutting method further. This could be done by cutting the slices of the fish muscle more accurately and uniformly. However, this could be a challenge due to the natural biological variation causing the fillet thickness to vary. One way to overcome the difficulty in cutting uniform slices could be introducing non-destructive methods to study the distribution of salt and moisture profiles such as ^{23}Na MRI.

NIR spectroscopy has been used in other food products to determine the acetic acid content (González-Sáiz, Esteban-Díez, Sánchez-Gallardo, & Pizarro, 2008). To explore the use of NIR spectroscopy further as a method for quality control of the marinated herring, more experiments of PLS prediction of the acetic acid content in the marinade as well as the herring fillet is needed. During this project, several determinations of the acid content both in the marinade as well as in the herring fillet were conducted. However, measurement errors occurred making the results non-reliable and no further exploration of determining the acid content using NIR was conducted. As both and adequate salt and acetic acid concentration are needed for achieving a safe marinated product, simultaneous, determination of these two parameters could prove to be very beneficial.

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Appendix

The manuscript for Paper I, II and III

Paper I

The influence of processing conditions on the weight change of single herring (*Clupea herengus*) fillets during marinating.

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ABSTRACT

One of the main issues in the manufacturing of marinated herring is the variation in yield, which in turn, is affected by the processing conditions and the variance in fat content. In the present work, we study these effects on individual herring fillets, with focus on the intermediate brining process. Brining time, brine concentration, marinade composition and storage time were varied. For brine concentrations 8%, 16% and 26%, the diffusion coefficient was $2.31 \times 10^{-9}\,m^2\,s^{-1}$, which was used for model development of salt change prediction in herring during brining. Conducting experiments on single fillets revealed a correlation between the fat content and the weight change after 35 days of marinating. The greatest change occurred within the first few days and only minor changes were seen during the storage period of up to one year. These results contribute to a better understanding of the herring marinating process, which can aid the optimization process in the industry.

1. Introduction

Marinated herring products are traditionally consumed in Northern European countries and manufactured by a process using a solution of salt and acetic acid in order to increase the ionic strength, and to decrease pH and hereby make the fish available for consumption most of the year (Rodger, Hastings, Cryne, & Bailey, 1984). The manufacturing of marinated herring varies from country to country; the herring fillets are submerged directly into a solution of salt and acetic acid in a typical German production, whereas in Denmark, an intermediate brining step is commonly used in the marinating process. The herring fillets are initially submerged in salt brine, then drained, and finally marinated in salt and acetic acid (Karl, Roepstorff, Huss, & Bloemsma, 1995). The marinating process affects the muscle proteins in fish, resulting in a change in the water content of the muscles, and consequently the weight change determiningthe product yield (Rodger et al., 1984). The product yield is an important aspect of profitable marinated herring production, and several factors potentially affect the product yield, such as the processing conditions and the variability of the raw material. However, little information exists about the intermediate brining in the marinating process and its effect on product yield. Studies on salt brining and how it influences product yield have been carried out for different fish species; such as cod (Barat, Rodriguez-Barona, Andres, & Fito, 2002; Thorarinsdottir, Arason, Bogason, & Kristbergsson, 2004), salmon (Gallart-Jornet et al., 2007a, 2007b) and herring (Birkeland, Sivertsvik, Neilsen, & Skåra, 2005). The effect of marinating on different quality properties has been studied for different fish species (Baygar, Alparslan, & Kaplan, 2012; Szymczak & Kołakowski, 2012; Szymczak, Kołakowski, & Felisiak, 2012; Topuz, 2016), however, these investigations did not include salt brining as an intermediate step in the marinating process and hence its effect on product yield. Studies of fish marinating have mainly been conducted batch wise (Birkeland et al., 2005; Szymczak & Kołakowski, 2012; Topuz, 2016), where it is often assumed that batches are homogenous groups, even though it is well known that variation within batches does occur. The fat content, which is often regarded as an important quality parameter for herring products varies substantially in herring fillets (Karl & Münkner, 2002). This can be due to the mixing of stocks and an uneven age distribution of the herrings within catches (Lane, Westgate, & Koopman, 2011; Nielsen, Hyldig, Nielsen, & Nielsen, 2005; Rajasilta, 1992). Moreover, there is also a seasonal variation in fat content due to changes in feed availability and water temperature. In addition, the fat content also follows the cycle of sexual maturation (Aidos, van der Padt, Luten, & Boom, 2002; Nielsen et al., 2005; Szlinder-Richert, Usydus, Wyszyński, & Adamczyk, 2010; Timberg, Koppel, Kuldjärv, & Paalme, 2011). Lastly, yearly differences in the fat content also occur (Aidos et al., 2002; Lane et al., 2011). Investigating the herring marinating process at batch level without taking the variation within the batch into account results in loss of valuable information concerning the effect of the biological variation in fat content on the process yield. In order to uncover the relation between process parameters, product yield and the raw material properties, it is therefore necessary to conduct studies at the level of

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Nomenclature

M_t	sample weight at time t (g)
M_0	initial weight of sample (g)
ΔM_t	weight changes of sample (*w*/w)
$\Delta M_{w,\ t}$	weight change of water (w/w)
$x_{w,\ t}$	weight fraction (w/w) of water in herring at time t
$x_{w,\ 0}$	initial weight fraction (w/w) of water in herring
wps	water phase salt (g/100 g water)
φ	osmotic coefficient
ν	number of NaCl ions
α	volume ratio of solution and herring fillet
q_n	non-zero positive roots of the transcendental equation
M_w	molar weight of water (kg/mol)
m	molality of NaCl in the solution (mol NaCl/kg H_2O)
L	characteristic dimension of the system (m)
$D_{App,s}$	apparent diffusion coefficient of salt in the water phase ($m^2\,s^{-1}$)
a_W	water activity
C_{wps}	salt concentration in water phase (kg salt ·(kg salt + kg $H_2O)^{-1}$)
$C_{wps,\ \infty}$	salt concentration in water phase in herring when in equilibrium with brine (kg salt·(kg salt + kg $H_2O)^{-1}$)
$C_{sb,\ 0}$	initial brine concentration (kg salt·(kg salt + kg $H_2O)^{-1}$)
$C_{sb,\ \infty}$	salt concentration in the brine in equilibrium with water phase of herring (kg salt·(kg salt + kg $H_2O)^{-1}$)

individual herrings. For that reason, the present study is unique, as the herring fillets were tagged and followed individually through the brining and marinating processes, which makes it possible to study the relation between the total fat content, the process parameters and the final yield.

A better scientific understanding of the marinating process at industrial level will make it possible to optimize the process, resulting in a better utilization of the raw material, a decrease in product loss and a higher financial outcome. The current processes used in industry are based on hands-on experience and knowledge passed on from previous employees, and not on a scientific understanding of the raw material and the process parameters (Voskresensky, 1965). Based on laboratory scale experiments, our aim was to investigate and explain the underlying mechanisms for the varying weight yield (outcome) of marinated herring products, with focus on the effects of the salt brining process. An additional outcome was also to provide a mathematical tool to model the brining process, that can be used for practical applications and assist the optimization process in the industry without the need of great computing power.

2. Material & Methods

2.1. Raw material and experimental design

Fresh herring butterfly fillets (*Clupea harengus*) were obtained from Skagerak Pelagic a/s (Skagen, Denmark). The fish were caught during different seasons (April, June and December) over a timespan of four years (2013–2015 and 2017). The fish were gutted, filleted (butterfly fillets), and immediately stored on ice in polystyrene boxes at 2 °C, then shipped to DTU, Lyngby, where the experiments were conducted within 2–3 days of arrival. The fresh butterfly fillets (with skin) were separated into two fillets; one fillet was numbered with a plastic tag using a fish-tagging gun (Avery Dennison, Mark III Pistol Grip Tool no. 10651) in order to track the weight of each individual fillet, and the other part of the butterfly fillet was frozen down (−40 °C) for chemical analysis of the fat content in the raw material.

2.2. Preparation of brine and marinade

Salt brine, defined as a solution of sodium chloride (referred as salt) in water, was prepared for the salt brining process of herring fillets. Saturated brine (26.5%) was prepared by dissolving 36 g of NaCl (salt) (Food-grade vacuum salt, ESCO, Hannover, Germany) per 100 g of water,to be used as a stock solution for brines with the concentrations of 8%, 13.6%, 16%, and 26% salt. Marinades, defined as a solution of acetic acid and salt (from the saturated stock solution) in water,were used for the herring marinating process. Marinades were prepared with varying concentrations of acetic acid and salt, the exact concentrations of which are specified in the text (Sections 3.4–3.6). The solutions were stirred and left overnight at 2 °C to ensure complete dissolution of the salt.

2.3. Brining

The fresh herringfillets were randomly divided into three groups, and brined in 8%, 16% or 26% salt solution with a brine-to-fish ratio of 1:1 for 24 h. At each sampling time, fillets were drained for 1–2 min using a sieve; two individual brine samples (app. 15–25 ml brine/marinade) and three fillets were taken from each bucket. Upon analysis, the brine was centrifuged at 3838 × *g* for 20 min at 5 °C to remove tissue parts and insoluble matter, and kept at −40 °C until analyses were carried out. Herring fillets were rinsed under running water - in order to avoid excess salt crystals on the flesh surface - before the chemical analysis were carried out. In the brining experiment, sampling occurred every hour during the 24 h of storage.

2.4. Brining and subsequently marinating

The butterfly fillets were randomly divided into groups and brined in a solution of 13.6% salt with a fish-to-brine-ratio of 1:1 (brining times are specified in the text) to simulate industrial settings. After the brining procedure the fillets were drained in a sieve and marinated in a solution of acetic acid and salt, with a fish-to-marinade ratio of 1:1 (the concentration of salt and acetic acid is specified in the text). All experiments were carried out at 2 °C. A part of the raw herring fillets were frozen for 2 months at −40 °C prior to marinating.

2.4.1. Registration of weight changes

The fillets were collected on a grid, which allowed the brine/marinade to drip off for 1–2 min before weight registration. The individual weight of the same 2–30 herring fillet was registered at each sampling time during the experimental run, which varied depending on the experimental set-up (specified in the figures). The change in weight and water is defined by Eqs. (1) and (2), respectively.

$$\Delta M_t = \frac{M_t - M_0}{M_0}\ [-] \tag{1}$$

$$\Delta M_{w,t} = \frac{M_t * x_{w,t} - M_0 * x_{w,0}}{M_0 * x_{w,0}}\ [-] \tag{2}$$

The weight change (ΔM_t) is defined as the difference between the weight at every time point divided by the initial weight (Eq. (1)), and the weight change of water ($\Delta M_{w,\ t}$) is defined as the difference in water content at every time point divided by the initial water content (Eq. (2)). M_t is the mass in grams at time *t*, M_0 is the mass in grams at $t = 0$ (fresh), $x_{w,\ 0}$ is the weight fraction of water at $t = 0$ and $x_{w,\ t}$ is the weight fraction of water at time *t*.

2.5. Description of the salt uptake behavior

The salt transport, from the brine into the water phase of the herring can be described by Fick's second law Eq. (3):

$$\frac{\partial C_{wps}}{\partial t} = D_{App,s}\frac{\partial^2 C_{wps}}{\partial x^2} \quad (3)$$

Where C_{wps} is the salt concentration in the water phase of the herring, (kg salt·(kg salt + kg H_2O)$^{-1}$), $D_{App,s}$ is the apparent diffusion coefficient of salt in the water phase of the herring ($m^2\,s^{-1}$), x is the distance (m) and t is the time (s). $D_{App,s}$ is considered constant and independent of the concentration of the diffusing distance, so the system is said to follow Fickian kinetics. Assuming that the water phase of the herring has the shape of a plane sheet, the brine solution has a limited volume, the solution/herring volume ratio is constant, and the region of the herring immediately adjacent to the brine interphase reaches equilibrium immediately after the contact, Eq. (3) can be integrated into the analytical solution given by Eq. (4) (Crank, 1979):

$$\frac{C_{wps} - C_{wps,0}}{C_{wps,\infty} - C_{wps,0}} = 1 - \sum_{n=1}^{\infty}\frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2}\, e^{-\frac{D_{App,s}\, q_n^2}{L^2}t} \quad (4)$$

Where $C_{wps,\,0}$ is the initial concentration of salt in the water phase of the herring,$C_{wps,\,\infty}$ is the concentration of salt in the water phase of the herring when it reaches equilibrium with the salt concentration in the brine, $L(m)$ is the characteristic dimension of the system (in this case, half of the thickness commonly referred to as semi-thickness of the herring fillet), α is the volume ratio of solution and herring fillet, and q_n are the non-zero positive roots of the transcendental Eq. (5):

$$tan\ q_n = -\alpha\ q_n \quad (5)$$

In order to describe the concentration evolution, some parameters are needed. $C_{wps,\,\infty}$ and L were observed or measured experimentally. The roots of the transcendental Eq. (5) were calculated using the fzero function implemented in Matlab 2015a (Mathworks, USA), while $D_{App,\,s}$ was determined by minimizing the sum of the square residuals between the experimental and the predicted salt concentrations for each of the 8%, 16% and 26% initial brine concentrations, according to Eq. (6). The minimization algorithm was based on the Levenberg-Marquardt algorithm, an optimization routine implemented in the lsqnonlin function, implemented in Matlab 2015a.

$$SSQR = \sum_{i=1}^{n}(C_{wps,exp} - C_{wps,pred})^2 \quad (6)$$

Calculation of the brine herring volume ratio:

Since the brine/herring mass ratio is 1:1, the brine/herring volume ratio can be calculated from the density ratio, α, as expressed in Eq. (7):

$$\alpha = \frac{V_{brine}}{V_{wph}} = \frac{m_{brine}\,\rho_{wph}}{m_{wph}\,\rho_{brine}} = \frac{\rho_{wph}}{\rho_{brine}} \quad (7)$$

Where V_{brine} and V_{wph}, is the volume of brine and water phase in herring, respectively, m_{brine} and m_{wph} is the mass of brine and water phase and ρ_{brine} andρ_{wph} is the density of the brine and water phase in herring, respectively. The density data for salt solutions in function of the salt concentration was gathered from Ionuţ Simion, Grigoraş, Roşu, and Gavrilă (2015), where it was assumed that the density of the water phase of the herring was the same as a salt solution. The value of α for each of the 8%, 16% and 26% datasets was calculated for the average densities of the brine and the water phase during the process.

2.6. Calculation of the water activity

The water activity is a measurement of the amount of free or unbound water of the system that is available for microbial activity. It was calculated as a function of the salt molality in the water solution, as expressed by Eq. (8) (Pazuki, 2005):

$$a_W = e^{-M_w \nu \varphi m} \quad (8)$$

Where a_W is the water activity, Mw is the molecular weight of water (kg/mol), ν is the number of ions (2 for NaCl), φ is the osmotic coefficient, and m is the molality of NaCl in the solution (mol NaCl kg H_2O^{-1}).The osmotic coefficient φ is a deviation of the behavior of a solution from ideality due to the ion concentration, as well as the water activity -it is a function of the molality of the system. This way, values of the osmotic coefficient in function of the molality of the system at 2 °Cwere extrapolated from data published by (Pitzer, Peiper, & Busey, 1984) and fitted using the function polyfit included in Matlab 2015a.

2.7. Chemical analyses

The saltcontent of brine fish samples was determined by titration with $AgNO_3$ (Titrator, 785 DMP Titrino with a magnetic stirrer, Metrohm).Dry matter was determined after heating the sample at 105 °C for 48 h, where a stable sample weight was achieved. The samples that were analyzed for salt content and dry matter are specified in the experimental set-up/result section. The total fat content was measured on fresh herring fillets on an individual basis (minced with skin on) by the standard method (Bligh & Dyer, 1959). Only herring fillets in ex. A-I were subjected to this analysis.

2.8. Data analysis

Statistical analyses were performed using GraphPad Prism 7 multiple *t*-test to find significant ($P < 0.05$) differences between the mean values of the groups at individual time points. As correlation test, the One-tailed Pearson Correlation ($\alpha = 0.05$) was used.

3. Results

3.1. Raw material batches

The fat content was measured for each individual fresh herring fillet in batches A-I. The average content of fat for all batches was 9.2 ± 5.8%, with individual fillets spanning the range from 1.2% to 24.7%. There was a between-batch variation where fish caught in December 2014 and June 2013 (batch A–D) had a higher fat content - 12.8 ± 4.2% - compared to those caught in April 2015 (batch E–G) and April 2017 (batch H and I), that had an average content of 3.5 ± 2.2% fat (Fig. 1). In order to study the different parameters, it was decided that batches with a similar level of fat content would be compared in order to limit the effect of the fat content. The batches of herring (1–3) used for brining (24 h) were not assessed for fat content.

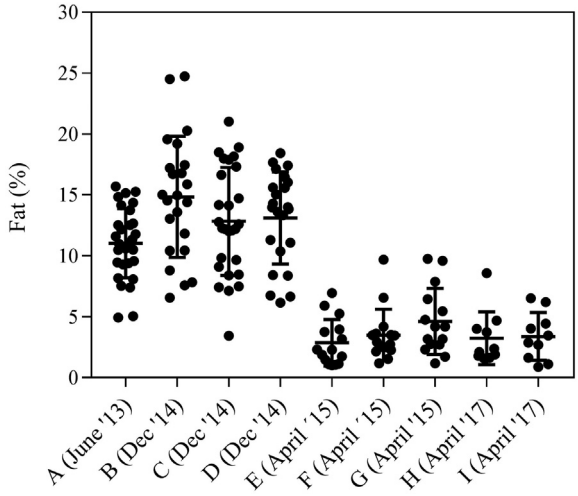

Fig. 1. The fat content of herring fillets in the batches A–I.

3.2. Brining

During the brining process, the brine and herring fillets exchange salt and water. Therefore, both the brine and the herring fillets will undergo changes in salt concentration, water concentration, and weight, thus directly influencing the safety and the yield of the final product. Therefore, an understanding of the salt and water transport processes involved would be helpful to gain a better control of the overall brining process. As seen in Fig. 2a, for all of the three brine concentrations (8%, 16% and 26%), the salt seems to be transferred from the brine into the herring until the salt concentration in the water phase of the herring is equal to the salt concentration in the brine. Table 1 displays the actual values after 24 h, and shows that the concentration in the brine and the water phase of the herring is not exactly the same. This may be due to the inherent experimental error, or to the existence of a partitioning effect between both phases.

The salt transport from the brine into the herring due to the concentration gradient between the brine and the water phase of the herring increases the ion concentration in the water phase of the herring, thus lowering its water activity, which initially was at its maximum of 1 (Fig. 2b). This means that, together with salt, water will also move from the brine into the herring in order to try to keep the water activity as high as possible. Since the water concentration in the brine is much higher than in the herring, and the latter can only take up water to a limited extent, the observed effect is that the water activity decreases in herring and increases in brine until both phases have reached the same level. This equilibrium water activity level was 0.975, 0.95 and 0.92 for 8%, 16% and 26%, respectively. As previously stated, this coupled salt and water uptake has a net impact on the fillet weight, which increases over time (Fig. 3a). The weight, as well as the salt and water concentrations, stabilized after around 9 h for fillets brined in the lowest concentration of salt (8%) as seen in Fig. 3a. This corresponds to approximately, the time at which salt concentration reaches equilibrium between the brine and the water phase of the fish,(Fig. 2a) as well as the time at which the weight change of water stabilizes (Fig. 3b). Fillets brined at the higher concentrations of salt (16% and 26%) increased in weight continuously during the whole brining procedure over 24 h (Fig. 3a), which corresponded to a continuous increase in water content (Fig. 3b). When brining at the highest concentration of salt (26%), the fillet weight increased only slowly in the early part of the brining process (Fig. 3a). This could be due to an osmotic loss of water (Fig. 3b) counteracting the initial increase in salt concentration in the muscle water phase (Fig. 2a). The change in weight and proportional water content, and the final salt concentration in brine and in fish muscle water phase is seen in Table 1, from which it is evident that fillets brined in 16% salt experienced the highest weight increase after 24 h, compared to fillets brined in 8% and 26% salt.

3.3. Modeling of the herring brining process

Fig. 2a represents the experimental average salt concentration development in the herring as a function of time, together with the predictions of the model, given by Eq. (4) and an average fillet semi-thickness of 0.7 cm. As stated in the materials and methods section, this equation describes the salt transport as diffusion of a species into a plane sheet from a solution of finite volume, with a speed determined by the apparent diffusion coefficient D_{App} and an asymptotic limit for the process given by the observed concentration of salt in the water phase of the herring that is in equilibrium with the brine. According to this description, the salt transport would be understood as the exchange between two pools of salt solutions with different concentrations. This description, of course, does not represent the previously described complexity of the salt and water exchanges between the brine and the herring but still describes quite satisfactorily, the average salt concentration development in the herring. The brining experiments with initial brine concentrations of 8%, 16% and 26% were used for calculating three values of D_{App}.

The apparent diffusion coefficient values (Table 1) do not present significant differences between the 3 brine concentrations of 8%, 16% and 26%, and the average value of $2.31 \times 10^{-9}\,m^2\,s^{-1}$is coherent with the few D_{App} data on herring that can be found in literature (Rodger et al., 1984), where diffusion coefficient values between 1.1 and $2.3 \times 10^{-10}\,m^2\,s^{-1}$ were found under other experimental conditions. As it can be seen in Fig. 2a, the average D_{App} can be used to describe the average salt concentration development in the water phase of the herring for any of the three initial concentrations of brine. This is a confirmation of what has already been observed experimentally: increasing the initial salt concentration of the brine has an effect on the equilibrium point of the system, but not in the process speed.

In order to use Eq. (4) to predict the average salt concentration in the herring, two parameters are needed: D_{App} (kinetic), and $C_{wps,\,\infty}$ (thermodynamic).As the differences on the diffusion coefficient are so

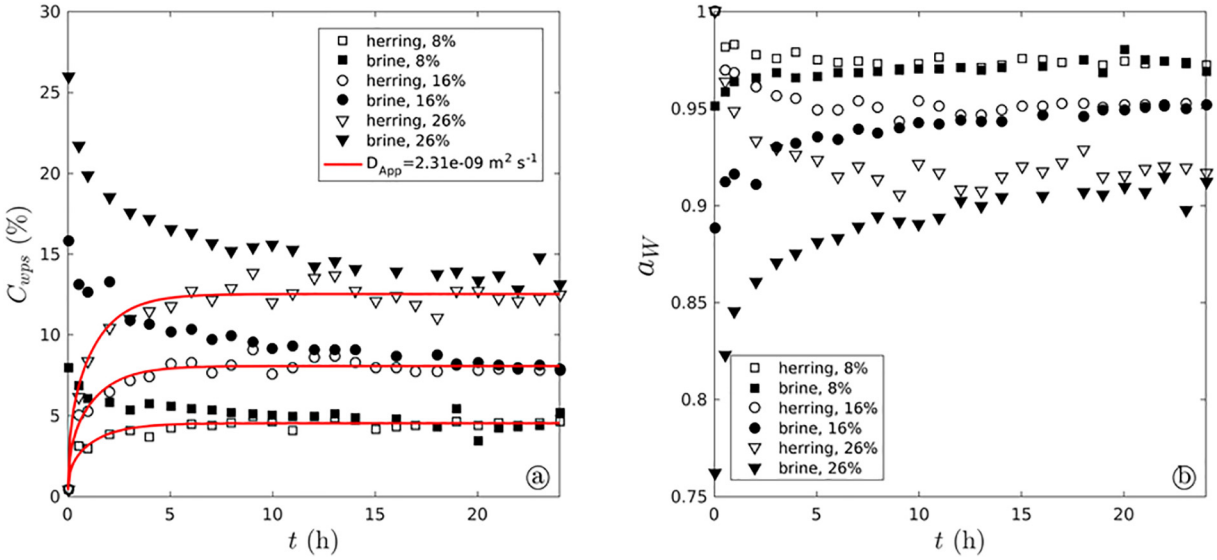

Fig. 2. Change in a) average salt concentration of the brine (n = 2) and the fish water phase (n = 3). The discrete points represent the experimental data, while the solid lines represent the predictions of Eq. (4), calculated with α and the average D_{App} from Table 1 and b) water activity during brining with 8%, 16% and 26% NaCl for 24 h.

Table 1
Change in salt concentration in brine and in fish muscle water phase, weight change, water content change and the apparent diffusion coefficient.

After 24 h of brining												
Brining conditions (% NaCl)	$C_{sb,\infty}$ (% NaCl)		$C_{wps,\infty}$ (% NaCl)		$\frac{C_{sb,\infty}}{C_{sb,0}}$		Weight change (%)		Water change (%)		α	$D_{App,s}$ $\times 10^{-9}$ ($m^2 s^{-1}$)
	Mean (n = 2)	SD	Mean (n = 3)	SD	Mean	SD	Mean (n = 2)	SD	Mean (n = 3)	SD		
8	5.2	0.6	4.7	0.3	0.59	0.07	13.5	0.2	28.6	0.0	0.99	2.71
16	7.9	0.2	8.0	0.0	0.54	0.04	19.3	3.1	26.0	0.0	0.98	2.25
26	13.1	0.1	12.6	0.5	0.54	0.04	15.6	2.8	13.3	0.0	0.97	1.96

Sb: salt brine, wps: water phase salt (g/100 g water), SD: Standard deviation, α: brine/herring volume ratio and $D_{app,s}$: apparent diffusion coefficient of salt.

insignificant, the proposed average of 2.31×10^{-9} $m^2 s^{-1}$ can be used regardless of the initial salt concentration of the brine. On the contrary, $C_{wps,\infty}$ is not the same for all the three studied cases, and thus, it becomes necessary to find a way to estimate it from the initial brine concentration. The brine concentration reaches equilibrium with the herring when 59% salt is left from the initial brine of 8%, and 54% salt is left after equilibrium for the initial brine concentrations of both 16% and 26%, which can be seen in Table 1. These differences may be attributed to the dependence between the initial and the equilibrium concentrations being exponential instead of linear, or, less likely, to the inherent experimental error. Although the mechanistic explanation of these dependencies lie outside the scope of this paper, it is possible to provide guidelines for the estimation of $C_{wps,\infty}$. This way, $C_{wps,\infty}$ will correspond to the 60% of the initial salt concentration of the brine for those below 8%, to the 56.5% for initial brine concentrations between 8 and 16%, and to the 54% for brine concentrations beyond 16%.

As it can be seen, the mechanistic meaning of the mathematical description given by Eq. (4), and specifically of the apparent diffusion coefficient, is limited, since it does not give any information about how the water activity gradient governs the water exchange that is suspected to be behind the observed weight changes, or of any partitioning effects between the equilibrium concentrations that may be observed in the experimental data. Doing so would require a more detailed model, which would require further research. Therefore, the equation, along with the average D_{App} and the proposed way to estimate $C_{wps,\infty}$ can be used to predict the salt concentration in the herring for direct practical purposes. However, we do not recommend trying to infer any understanding about how the system behaves the way it does.

Eq. (4), together with the average D_{App} has been implemented in a spreadsheet and made publicly available together with the electronic version of this article (see section supplementary data).

3.4. Marinating

Herring fillets increased in weight during brining due to uptake of salt and water. However, in the subsequent marinating procedure, the fillet weight was reduced. Herring fillets marinated in 6% and 9% acetic acid acted similarly and had a faster and bigger weight decrease compared to fillets marinated in 3% acetic acid ($P < 0.05$), as seen in Fig. 4a. The main weight change of the fillets occurred within the first three days of marinating at all the acetic acid concentrations (Fig. 4a), and the fillets marinated in 3% acetic acid only lost 5% in weight, compared to a 10–11% weight decrease for fillets marinated in 6% and 9% acetic acid.

The manufactures often store the marinated herring fillets longer than the minimum storage time of 35 days due to changes in demand, and during this storage period the herring products tende to decrease in weight. Fillets from batch A (results not shown) were stored up to 365 days in the marinade, and after day 3 (2 days in brine and 1 day in marinade), where the weight decrease was 10% of the initial weight, only minor changes in the weight were observed as the weight decrease was 13.3% after 35 days and 13.5% after 365 days. In batch F the fillets were stored for 112 days with an additional weight loss of 2% compared to day 35 (Fig. 4a).

3.5. Effect of brining in the marinating process

The effect of brining time, i.e. the change in weight after the brining

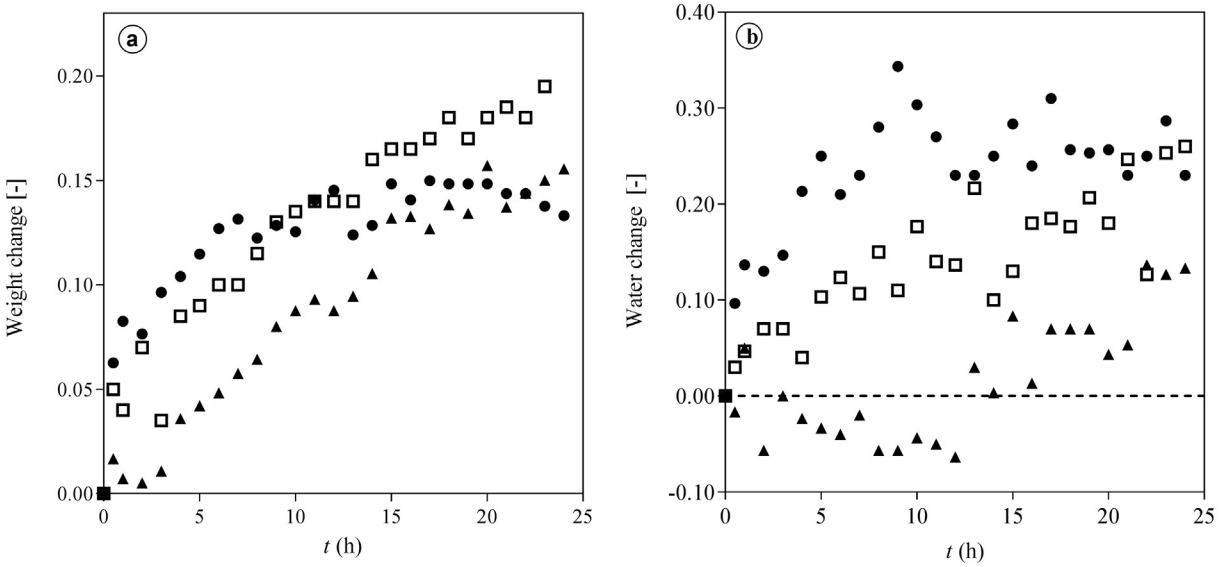

Fig. 3. Change in a) fillet weight (ΔM_t) and b) water content ($\Delta M_{w,t}$) during brining in 8% (●), 16% (□) and 26% (▲) NaCl solution (average values of n = 2).

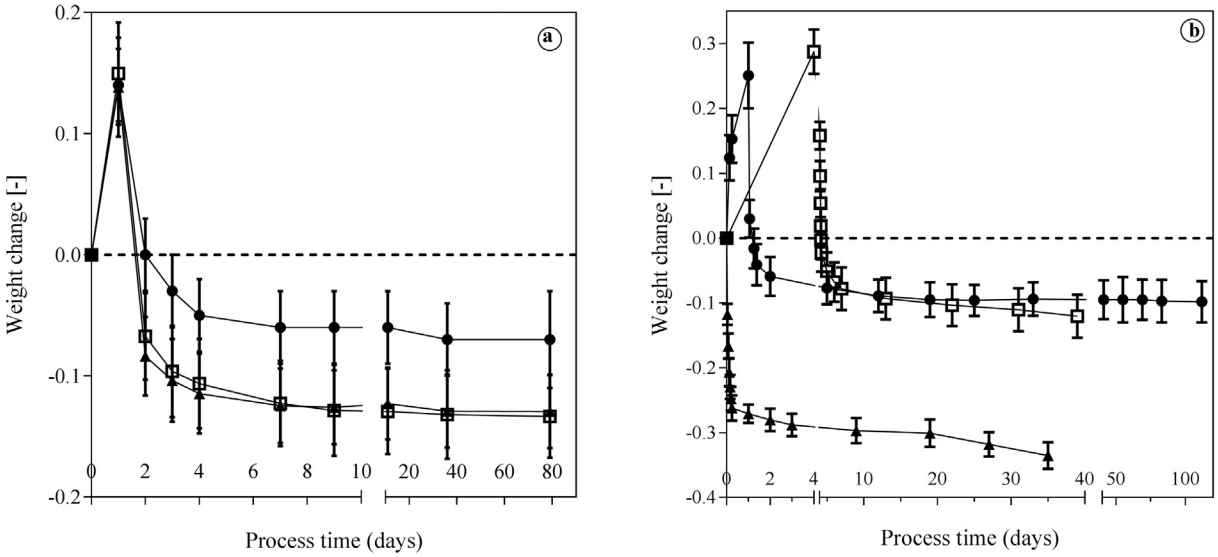

Fig. 4. Change in fillet weight during brining in 13.6% NaCl a) for 24 h and marinating in 4.3% NaCl with 3% (batch B) (●), 6% (batch C) (□) and 9% (batch D) (▲) acetic acid and b) for 24 h (batch F) (●), 4 days (batch H) (□) or no brining process (batch I) (▲) and marinating in 5.4% NaCl and 5.8% acetic acid.

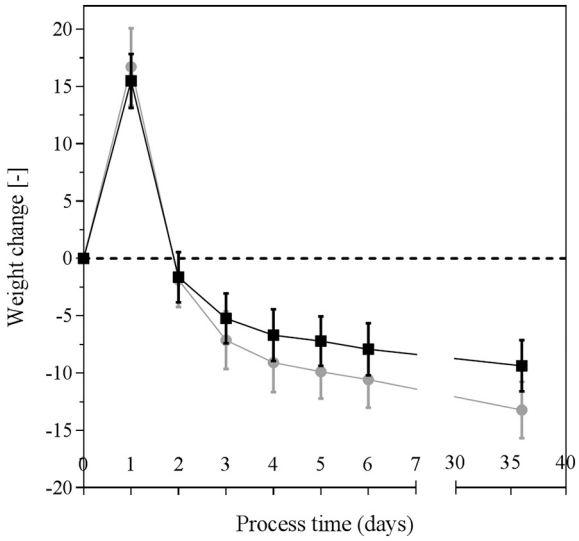

Fig. 5. Change in fillet weight during brining (13.6% NaCl) and marinating (5% NaCl and 6.6% acetic acid) of fresh (■) and frozen herring (●). Herrings were caught around July 2014 and frozen for app. 2 months at −40 °C. Fresh herrings were caught in end August 2014.

procedure, before the subsequent marinating process was investigated by comparing fillets that were put directly into the marinade (batch I) with fillets brined either 24 h or 4 days before the marinating (batch F and H) (Fig. 4b). During the first three days of marinating, the weight of the directly marinated fillets decreased with 28.8% and ended with a 33% decrease from the initial weight after 35 days of marinating. Compared to this, the 24 h brined fillets had a weight loss of 7.8% and 12% after 3 and 35 days in the marinade, respectively.

The influence of extending brining time by 24 h on the final weight loss after marinating showed that the fillet weight gain after brining for 24 h and 4 days was slightly different ($P = .052$), with a weight increase of 25 ± 0.05% after 24 h and 29 ± 0.03% after 4 days (Fig. 4b). However, there was no difference in the weight decrease during the subsequent marinating procedure, where a weight reduction of approximately 7% from the initial weight was seen after three days of marinating for fillets brined for 24 h and 4 days.

3.6. Effect of fat content and treatment of the raw material

We compared the fat content of each individual herring fillet (one part of the butterfly fillet) with the total weight change of the marinated fillet (the other part of the butterfly fillet) after 35 days of processing (typical minimum storage time in the industry) for the batches A-I, in order to investigate the importance of the fat variation in the raw material. Our results showed that the weight loss during the marinating process (35 days) increased significantly ($P < 0.05$) with decreasing fat content for 5 of the batches (B–F), and there was a tendency for a similar correlation for herrings in batch I ($P = 0.08$). This implied that fatty herrings had less water to lose during the marinating process, which would be beneficial for the manufacturers in achieving a higher product yield.

The weight change during the marinating procedure is not only related to the raw material composition but also the handling of the raw material beforehand. Fresh herring fillets have mainly been studied in this work, but commonly the industry also use frozen material due to changes in demand and raw material availability. For that reason, the weight changes during the marinating procedure were investigated in pre-frozen herring fillets and compared to fresh fillets originating from the same batch. There was no significant difference in the weight gain obtained during brining between fillets which were previously frozen and fresh fillets. However, during the marinating process, the fish that was previously frozen decreased in weightmore quickly than the fresh fillets, with a significant difference between the groups from day 4 and onward ($P < 0.05$) (Fig. 5).

4. Discussion

Conducting brining and marinating experiments using individual herring butterfly fillets enables the explanation of the varying weight yield during processing and its correlation to the fat content of the raw material. Analyzing the fat content on each single herring fillet revealed a great within-batch and between-batch variation.A change in yield during brining and marinating of herring fillets is a result of the multiple transport of water, salt, acetic acid, salt-soluble proteins, smaller peptides and other nitrogenous compounds (Szymczak, 2011; Szymczak & Kołakowski, 2012). Our results showed a correlation between the fat content of raw herring fillets and their subsequent weight change after 35 days of marinating. The herring muscle mainly consists of fat, proteins and a liquid phase in which water is the solvent, and salt and

solubilized proteins are the major constituents. There is a strong linear relationship between the water content and the total fat content in herring (Nielsen et al., 2005). The salt uptake is affected by the amount of fat content present in the fish: either by replacing the aqueous phase that serves as a path for transfer, or by acting as a physical barrier (Gallart-Jornet et al., 2007b). We suggest the explanation of the correlation of the weight loss and the total fat content in herring during the marinating procedure is that the fat acts as a passive component in the transfers mechanism, and it is the amount of water phase present in the muscle that determine how much water there is available for diffusional exchange with the surrounding marinade. The weight change during the marinating procedure is not only related to the raw material composition, but also the handling of the raw material beforehand. Freezing of the fish before processing causes damage of cellular membranes and proteins (Szymczak, 2011). Our results showed that a lower yield was observed for the pre-frozen fillets compared to fresh fillets during the marinating process (Fig. 5), which could mainly be explained by the lower water holding capacity due to the change in proteins during freezing.

Throughout this study, a brine-fish-ratio of 1:1 has been used, as low brine-to-fish ratios are most common in industrial processes of fish brining and marinating., Therefore, investigations conducted at low brine-to-fish ratios are relevant. Using excess brine in the industry may also be a disadvantage for both economic and practical reasons. The brine-to-fish ratio is related to the change in concentration of salt in the fish and in the brine/marinade, the water content of the fish, and hence the change in yield during processing. The brine concentration decreased rapidly in the beginning of the brining process (after 10 h) to 5.1%, 9.2% and 15.6% from the initial concentrations of 8%, 16% and 26% (Fig. 2a). These could be a result of the low brine-to-fish ratio (1:1) that makes it sensitive to the salt and water flux between the brine and the fish muscle (Birkeland et al., 2005). As see in Table 1, only minor changes in brine concentration were observed at the end of the brining process (24 h), which emphasize the importance of a high sample frequency in the beginning of the process. Our results showed that the salt concentration in the muscle water phase increased with increasing brine concentration, from 4.7% at 8% brine concentration to 12.6% at 26% brine concentration, respectively. Equilibrium between the salting medium and fish water phase was reached within 24 h of brining for all the three initial brine concentrations (Fig. 2a). Brining with higher concentration of salt (26%) resulted in a loss of water in the beginning of the process (Fig. 3b). This could be explained by protein denaturation mainly at the surface resulting in reduced water holding capacity (Gallart-Jornet et al., 2007b). The fish muscle properties change during the salting process because of the interaction of salt and the protein matrix, which changes the water holding capacity. The brine-to-fish ratio (1:1) used in this study affects the concentration in the brine and fish, which consequently affects the changes in fillet weight during the brining process. An increase in weight was observed for all three brining concentrations and was 13.3%, 19.3% and 15.6% for brine concentration of 8%, 16% and 26%, respectively (Table 1). Fillets salted in 8% brine reached a weight equilibrium value within 8–9 h of the process (Fig. 3a), which is supported by the equilibrium in salt and water flux (Figs. 2a and 3b). However, it seems that the fillets salted in 16% and 26% brine reach concentration equalization at the end of the process, but the weight seems to increase throughout the process and possibly reach equilibrium later on (Birkeland et al., 2005; Barat, Rodríguez-Barona, Andrés, & Fito, 2003). A decrease in weight during brining with 25% salt is observed for cod and salmon when using a brine-to-fish ratio of (3:1) (Gallart-Jornet et al., 2007b), emphasizing the effect of the brine-to-fish ratio. The maximum protein swelling occurs at salt concentration of 1 M (~5.8% salt) (Erikson, Veliyulin, Singstad, & Aursand, 2006; Gallart-Jornet et al., 2007a; Thorarinsdottir, Arason, Sigurgisladottir, Valsdottir, & Tornberg, 2011), which is close to the final concentration in fillets that had the highest increase in weight during the marinating procedure (Table 1; initial brine 16%). The brine-to-fish-ratio affects the concentration of salt and water in the fish muscle and consequently the fillet weight (Capaccioni, Casales, & Yeannes, 2011; Rodger et al., 1984).

During the subsequent marinating procedure, the acetic acid diffuses into the herring muscle, which lowers the pH, causing protein denaturation and lower water absorption (Szymczak, 2011). The brining process causes an increase in herring weight due to the uptake of salt and water affected by the brine-to-fish ration (1:1). In the following marinating procedure, the fillet weight decreases with an increasing acid concentration. However, fillets marinated in 6% and 9% acid acted similarly in relation to weight change (Fig. 4a). Similar results have been reported for tunny fish (Topuz, 2016) and herring fillets (Szymczak, Kołakowski, & Felisiak, 2015), where the product yield gradually decrease with the increase of acid concentration. Our results show that the greatest change in weight occurred within the first three days of marinating, which most likely was due to loss of water and water-soluble compounds. Only minor changes in weight is seen during storage above 35 days (Fig. 4a and b), which could be explained by the continuous diffusion of proteins, other nitrogen fractions, lipids, free fatty acids and minerals from herring muscle to the marinade (Szymczak & Kołakowski, 2012). The essential factor for preservation is the acid, but too much acid will make the fillets too soft, so there is a need for salt (McLay, 1971). If only acetic acid was present in the marinade, the muscle pH will be on the acidic side of the isoelectric point and electrostatic repulsion, causing an “open” structure. This will result in an increase in water holding, but with the presence of salt, the repulsive charges are shielded from one another and cause a decreased water holding capacity. Therefore the extent of the effect of both salt and acid is related to the concentration of the two (Rodger et al., 1984).

The mechanistic explanation of the salt and water exchanges between the brine and the herring is given on the basis of the brining experiments that last 24 h. It is yet to be found out if this description is able to fully represent the water and salt exchanges that occur during thefollowing 35 days marinating period as used for batches A-I. The model conducted in this study is appropriate for practical application that pursues only to predict the average concentration of salt in the herring water phase as a function of time. This is stated on the basis of a single apparent diffusion coefficient being able to describe the aforementioned concentration evolution, regardless of the initial brine concentration. Due to model simplicity, i.e. excluding the influence of the water transport that leads to weight change, it is limited to the prediction of the salt concentration development in the fish and not in brine.

5. Conclusion

Optimization of the marinating process is important for the industry. Their production is primarily based on know-how. A marinating procedure similar to the industry was studied in a controlled lab environment, and showed the existence of a great within-batch variation and the effect of different marinating conditions on weight change/yield in herring fillets, which was possible due to the investigation on single fillets. Experiments were conducted in different seasons and different years revealing the seasonal and yearly variation. It was found that the fat content had a significant effect on the weight change during the marinating procedure and the greatest change occurred in the first few days of marinating, and only smaller changes are seen during storage of up to one year. The presented model, which is an analytical solution of Fick's second law, is appropriate for a direct practical application that seeks only to predict the average salt concentration in the water phase of the herring as a function of time. These results contribute to a better understanding of the herring marinating process, which can aid the optimization process in the industry.

Declaration of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2018.03.055.

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Paper II

Mechanistic modelling of the coupled salt and water transport in herring during brining and curing

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Mechanistic modelling of the coupled salt and water transport in herring during brining and curing

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Abstract

Salting of fish tissue triggers a water movement to equilibrate the activity gradient. A mechanistic model that can predict the development of salt and water concentration as well as the water activity distributions, was validated by developing an experimental methodology that allows the access to the salt and water concentration distributions at any stage of the process. The model succeeded on offering reasonable predictions, with average RMSEs of 0.019 and 0.033 $kg \cdot kg^{-1}$ for the salt and water concentration distributions, and 0.022 for the water activity; as well as a mathematical framework that is independent of the salting conditions (e.g. brining, curing), which helps to understand why the system behaves the way it does. Additionally, it allows the establishment of a tool to have a better control of the salting time in order to ensure both organoleptic and safety requirements.

Keywords: salt transport; herring; mechanistic modelling; food safety; process optimization

1. Introduction

Salting of herring is an old preservation method that has been used for centuries in the North European countries (Birkeland, Sivertsvik, Nielsen, & Skåra, 2005). Differences in salting methods, salt concentrations and ripening time have resulted in many variations of salted herring products (Nielsen, 1995), but it is still commonly used as an intermediate step in marinating (Karl, Roepstorff, Huss, & Bloemsma, 1995; Rodger, Hastings, Cryne, & Bailey, 1984). Traditional barrel salted herring products are produced with the use of a combination of dry salting and subsequently addition of saturated brine (Gringer, 2015), whereas for marinated herring products salt concentrations around 10 % or above are commonly used in the pre-brining (Szymczak, Kołakowski, & Felisiak, 2012). Common to all of these different salting processes is that a water movement is triggered as a consequence of the salt uptake (Gallart-Jornet et al., 2007).

Understanding the salting kinetics is of great importance for industrial processes in order to develop new products and optimize existing processes conditions. To this respect, mathematical models that are based on mechanistic principles can provide a better control of process variables such as brine concentration and immersion time (Andreetta-Gorelkina, Gorelkin, & Rustad, 2016; Zhang, Xiong, Liu, Xu, & Zhao, 2011). The mechanistic models are easily adapted to changes in the process conditions since, differently from data-driven models, they offer an explanation about why the aforementioned variables interact the way they do. As those models are generally based on the continuity equations, their development requires the determination of the transport properties under relevant conditions.

Fish muscle is a complex food type whose properties evolve with time. For example, during salting, the interaction of salt and the myofibrillar proteins affect the water holding capacity and chemical potential of the muscle, leading to swelling or shrinkage (Gallart-Jornet et al., 2007). In addition, fat

as well as the skin are also known to influence the salting process and be a limiting factor for salt and water diffusion (Gallart-Jornet et al., 2007; Rodger et al., 1984). The transfer of salt is believed to be governed by the concentration gradient and the water transfer by the osmotic pressure between the muscle and the salting medium (Barat, Rodríguez-Barona, Andrés, & Fito, 2003; Thorarinsdottir, 2010).

Most salting studies in fish products concern the transport of salt and not the coupled transport of both salt and water (Andreetta-Gorelkina et al., 2016; Rodger et al., 1984; Zhang et al., 2011). Additionally, many of these studies (Andreetta-Gorelkina et al., 2016; Wang, Correia, and Tang, 1998; Wang, Tang, & Correia, 2000) are based on global concentration methods, or in other words, on the development of the average concentration over time until equilibrium is reached (Akköse & Aktaş, 2015). For example, the model by Zugarramurdi and Lupin, which has been widely used to describe the water and salt kinetics (Bellagha, Sahli, Farhat, Kechaou, & Glenza, 2007; Corzo, Bracho, & Rodríguez, 2015; Czerner & Yeannes, 2013), can predict the average salt and the water concentrations at any stage of the process, but presents the salt and the water transport as independent phenomena, thus not providing any explanation about how the salt uptake triggers the water transport.

Local concentration methods, on the other hand, provide much more information, as they give access to the distribution of salt and water inside the fish muscle and how it develops over time (Boudhrioua, Djendoubi, Bellagha, & Kechaou, 2009). Local concentration profiles of salt have been investigated in a non-destructive way by ^{23}Na MRI in meat (Vestergaard, Andersen, & Adler-Nissen, 2007) and in salmon and cod (Gallart-Jornet et al., 2007) and by destructive methods where the concentration profiles were quantified by slicing the fish samples for sardines (Boudhrioua et al., 2009). In this study, the coupled salt and moisture transfer in the herring muscle was investigated under different salting conditions; brining at 16% and 26% (w/w) and curing (dry salting). This required the development of an experimental methodology that allows to measure the salt and water concentration

profiles at different stages of the process. Additionally, the mechanistic forward osmosis model developed by Martinez-Lopez (Martinez-Lopez, 2018)was applied in order to predict the concentration distributions. Results show an acceptable predictability, while offering an explanation about the phenomenon triggering the water movement to the salt uptake.

2. Materials & methods

2.1 Raw material

Fresh herring fillets (*Clupea harengus*) were obtained from Skagerak Pelagic a/s (Skagen, Denmark). The fish was caught during November 2016 and gutted, filleted (butterfly fillets) and immediately packed with ice in polystyrene boxes at 2 °C and transported to DTU, Lyngby, and stored at 2 °C until the experiments were carried out within 1-2 days of arrival. The fresh butterfly fillets with skin were separated into two fillets, and 1×2 cm were cut from each fillet (near the head region) and analysed for initial moisture and salt content on individual samples. A fish-tagging gun (Avery Dennison, Mark III Pistol Grip Tool no. 10651) was used for tagging each fillet with a plastic tag in order to track each individual fillet during salting.

2.2 Salting conditions and procedure

Herring fillets were salted using dry salt, 16 % or 26 % (w/v) salt brine. Saturated brine (26.5 %) was prepared by dissolving 36 g of salt (Food-grade vacuum salt, ESCO, Hannover, Germany) per 100 mL of water, which was used as a stock solution for brines with the concentrations of 16 % and 26 % salt. The salting procedure was carried out at 2 °C. For dry salting, herring fillets were placed with skin-side down in trays with both sides covered with salt. The trays were covered with plastic film. More salt was added during the storage period to ensure a continuous supply of salt. The fillets were left for 6 h, 24 h and 48 h before the fillets were removed from the dry salting. For the brining procedure, three fillets were immersed in excess brine (brine-to-fish ratio of 17:1) (16 % or 26 % salt

in 5 L plastic buckets with lids). This was done so the brine concentration throughout the experiment can be safely assumed as constant. It was ensured that the fillets were covered with brine and that all surfaces were in contact with the salting medium. The containers were left for 6, 24 and 48 h for the 16 % brine and 1, 6, 24 and 48 h for the 26 % brine before fillets were removed from the bathing medium. Three fillets were removed from the experimental set-up at each sampling point for moisture and salt, rinsed under running water to remove excess salt crystals on the surface, and carefully padded dry. The fillets were weighed and cut into samples of approximately 3 x 3 cm (exact dimensions were registered) 2.5 cm from the head region and immediately frozen using liquid nitrogen until boiling stopped. The samples were stored at -40 °C until further analyses.

2.3 Slicing procedure

Slicing of the frozen samples was conducted on a microtome (Reichert-Jung 2800 Frigocut) at the temperature range -28 °C to -38 °C depending on the salt concentration in the fish. Samples were mounted on a specimen block with Tissue-Tek (Sakura, NL) and placed in the freezing cabinet to equalize the temperature in the sample. Samples were sliced from the surface of the fillet towards the skin side (Figure 1) perpendicular to the direction of diffusion. The exact thickness of each slice was measured on a TA-XT Texture Analyzer (Stable Micro Systems) using a flat-ended cylinder (P10) and the sample thickness were found from the point where the trigger force (0.002 kg) was reached. The number of slices and their thickness depended on the thickness of the fillet that was sliced, but it consisted typically on 10 slices of thicknesses between 0.6 and 1.5 mm. Salt and moisture content was measured on each slice.

2.4 Determination of salt and moisture content

Salt and moisture was determined on each slice from the salted fillets. Salt (NaCl) content was determined by titration with $AgNO_3$ (Titrator, 785 DMP Titrino with a magnetic stirrer, Metrohm). Moisture content was determined after heating the sample at 105 °C for 48 h until a stable sample weight was achieved.

2.5 Assessment of the initial salt concentration and moisture distribution

The initial salt and moisture content of each fillet was determined from a of 1 × 2 cm sample, taken from near the head region and sliced. The salt concentration was found to range between 0.04 and 0.05 kg kg^{-1}, and to be homogeneously distributed throughout each fillet. The moisture was found to vary significantly between fillets, with average contents ranging between 0.62 and 0.81 kg kg^{-1}. Additionally, the water was found to not be evenly distributed throughout the samples, ranging between an 89 % of the average water content on the region close to the skin, to a 110% of the average water content close to the center. The initial water distributions were smoothed using a polynomial function for modelling purposes.

2.5 Calculation of water activity

The water activity is a measurement of the amount of free or unbound water of the system. It was calculated as a function of the salt molality in the water phase of the herring, as expressed by equation 1 (Pazuki, 2005):

$$a_W = e^{-M_w \nu \varphi m} \quad (1)$$

Where a_W is the water activity, M_w is the molecular weight of water (0.018 kg · mol^{-1}), ν is the number of ions (2 for NaCl), φ is the osmotic coefficient, and m is the molality of NaCl in the water phase of the herring (mol NaCl kg H_2O^{-1}). The osmotic coefficient φ is a deviation of the behavior of a solution from ideality due to the presence of ions. Similarly to water activity, the osmotic coefficient is also a function of the molality of the system. This way, values of the osmotic coefficient in function

of the molality of the system at 2 °C were deduced from Pitzer, Peiper, & Busey (1984) and fitted using the function polyfit included in Matlab 2015a. For numerical reasons, the molality was replaced concentrations of salt and water in kg · kg^{-1}, by the change of variable given in eq. 2:

$$\ln a_w = -M_w \cdot \varphi \cdot m = -\varphi \cdot v \frac{C_s \cdot M_w}{C_w \cdot M_s} \quad (2)$$

Where C_s is the salt concentration (kg · kg^{-1}), C_w, is the water concentration in wet basis (kg · kg^{-1}) and M_s is the molecular weight of salt (0.0583 kg·mol^{-1}).

2.6 Calculation of salt activity

The thermodynamic activity of salt is a measurement of the effective concentration of a solute in a solution. The thermodynamic activity of salt, in function of its molality can be expressed as in equation 3 (Canagaratna & Maheswaran, 2013).

$$a_s = \gamma_m \frac{m}{m^-} \quad (3)$$

Where a_s is the thermodynamic activity of salt, γ_m is the activity coefficient of salt in an aqueous solution in function of the molality, m is the molality (mol NaCl kg H_2O^{-1}), and m^- is the standard molality (5.55 mol NaCl kg H_2O^{-1}). The values of the mean NaCl activity coefficient in function of the molality of the system at 2 °C were extrapolated from data published by (Pitzer et al., 1984) and fitted using the function polyfit included in Matlab 2015a.

2.7 Parameter determination

The transport properties of salt and water (table 1) were determined by minimizing the sum of the square residuals between the experimental and the predicted salt and water concentrations for each of the salting conditions. The minimization algorithm was based on the Levenberg-Marquardt

algorithm, an optimization routine implemented in the lsqnonlin function, implemented in Matlab 2015a.

$$SSQR = \sum_{i=1}^{n} (C_{exp,i} - C_{pred,i})^2 \quad (4)$$

Where C_{exp} is the experimental determined and C_{pred} is the predicted salt or water concentration.

The root mean square errors (RMSEs) were used to assess the performance of the model:

$$RMSE = \sqrt{\frac{SSQR}{N}} \quad (5)$$

Where N is the number of points of each concentration profile.

3. Theory

The forward osmosis model described by (Martinez-Lopez, 2018)has been adapted here to the particularities of herring. The model was implemented using the Method of Lines and solved by Matlabs built-in solver ode15s with an explicit method. Since the mass concentration (kg m^{-3}) and the mass fraction (kg kg^{-1}) can be considered directly proportional to each other (Ionut-Simon et al. 2015), the model makes use of mass fractions of salt and water instead of mass concentrations for practical reasons.

3.1 Salt transport

During dry salting, the salt crystals are solubilized at the fish surface and diffusing through the muscle. Differently, during brining a binary solution of salt and water surrounds the fish muscle with a constant concentration. In any case, the single-dimensional transport of salt throughout the herring can be described by equation 6:

$$\frac{\partial C_s}{\partial t} = D_s \frac{\partial^2 C_s}{\partial x^2} \quad (6)$$

Where C_s is the salt mass fraction in wet basis (kg · kg $^{-1}$), D_s is the diffusion coefficient of salt in the herring muscle (m^2 s^{-1}), x is the position in the direction of diffusion (m), and t is the time (s). Notice that equation 6 assumes that the diffusion coefficient of salt is constant at any time.

At $x = 0$, or in the salting environment/muscle interface, the salt flux is directly proportional to the difference between solubility limit of salt in the muscle $C_{s,m,\infty}$ (kg · kg^{-1}), and the concentration at $x = 0, C_{s,x=0}$ (kg·kg^{-1}). This assumption is valid while the salt concentration in the interface is lower than the solubility limit, which leads to eq. 7:

$$-D_s \frac{\partial C_s}{\partial x}\Big|_{x=0} = k_{s,m}\left(C_{s,m,\infty} - C_{s,x=0}\right) for\ 0 < t, C_{s,x=0} < C_{s,m,\infty} \quad (7)$$

Where $k_{s,m}$ is the mass transfer coefficient between the salting medium and the fish muscle (m·s^{-1}). For simplification purposes, when the interface has reached $C_{s,m,\infty}$, equation 7 can be replaced by:

$$C_s = C_{s,m,\infty}, for\ x = 0 \quad (8)$$

At $x = L$, there is an interface constituted by a very thin and permeable skin that is in direct contact with the salting medium, and an immediately adjacent layer of impermeable fatty muscle. This interface, probably due to the layer of fatty muscle, does not allow salt to penetrate into the system up to an observable extent. Therefore, the salt flux through this interface can be assumed equal to zero, which leads to the equation 9:

$$D_s \frac{\partial C_s}{\partial x}\Big|_{x=L} = 0 \quad (9)$$

When the salt activity is equal throughout the system, which in practical terms is the same as if the water phase of the herring muscle becomes saturated with salt, equation 9 can be replaced by equation 10 for simplification purposes.

$$C_s = C_{s,s,\infty} \text{ for } x = L \quad (10)$$

Since it is known beforehand that $C_{s,m,\infty} > C_{s,s,\infty}$, the moment at which equation 9 can be replaced by equation 10, the system will be very close to the steady state. At the beginning of the process, the salt concentration can be assumed homogeneously distributed throughout the fillet and equal to $C_{s,0.}$ Therefore:

$$C_s = C_{s,0} \text{ for } t = 0, \ \forall x$$

3.2 Water transport

Differently from salt, whose driving force is the concentration gradient, the water transport is driven by the water activity gradient. This way, the single dimensional transport of water is given by equation 11 (Bird, Stewart, & Lightfoot, 2007) :

$$\frac{\partial C_w}{\partial t} = D_{a_w} \cdot \frac{\partial}{\partial x}\left(C_w \cdot \frac{\partial \ln a_w}{\partial x}\right) \quad (11)$$

Where C_w is the water concentration in wet basis, ($kg \cdot kg^{-1}$), D_{a_w} is the non-ideal water diffusion coefficient ($m^2 \cdot s^{-1}$) which, similarly to D_s in the salt transport, is considered constant throughout the process, and $\ln a_w$ is natural logarithm of the water activity. As it can be seen, since the driving force is the activity gradient, the water transport is in the practice, also dependent on the salt transport. In the boundaries, the flux is proportional to the difference between the water activity in the muscle boundary at time t, and the water activity at equilibrium state. Given this, the boundary condition for the muscle interface can be written as:

$$-D_{a_w} \cdot C_{w,x=0} \left.\frac{\partial \ln a_w}{\partial x}\right|_{x=0} = k_{a_w,m} \cdot C_{w,x=0}\left(\ln a_{w,m,\infty} - \ln a_{w,x=0}\right), \quad (12)$$

$$\text{for } \ln a_{w,m,\infty} < \ln a_{w,x=0}$$

Where $k_{aw,m}$ is the non-ideal water mass transfer coefficient between the salting medium and the fish muscle ($m \cdot s^{-1}$), $C_{w,x=0}$ is the concentration of water at the muscle interface, $\ln a_{w,x=0}$ is the natural

logarithm of the water activity at the muscle interface and $\ln a_{w,m,\infty}$ is the natural logarithm of the water activity at the muscle interface. Since brine has a very significant water concentration, water may flow through this boundary either from the herring into the brine, or from the brine into the herring, as a function of the value of $\ln a_{w,x=0}$. This is not the situation during dry salting, since the salting environment is a pure salt phase. This way, when the water activity in muscle interface reaches the minimum $\ln a_{w,m,\infty}$, boundary condition (13) is restricted to $\ln a_{w,m,\infty} < \ln a_{w,x=0}$, and when $\ln a_{w,m,\infty} = \ln a_{w,x=0}$, the flux stops shortly until, because of the internal water movement, towards the boundary, $\ln a_{w,m,\infty} < \ln a_{w,x=0}$ again. This is expressed by equation 13:

$$-D_{a_w} \cdot C_{w,x=0} \left.\frac{\partial \ln a_w}{\partial x}\right|_{x=0} = 0\,, when\ \ln a_{w,m,\infty} = \ln a_{w,x=0} \tag{13}$$

In an analogous way, the boundary conditions for the skin is given:

$$D_{a_w} \cdot C_{w,x=L} \left.\frac{\partial \ln a_w}{\partial x}\right|_{x=L} = k_{a_{w,s}} C_{w,x=L}\left(\ln a_{w,s,\infty} - \ln a_{w,x=L}\right), \tag{14}$$

$$for\ \ln a_{w,s,\infty} < \ln a_{w,x=L}$$

Where $k_{a_w,s}$ is the non-ideal water mass transfer coefficient between the salting medium and the fish skin ($m \cdot s^{-1}$) due to the activity gradient, $C_{w,x=L}$ is the concentration of water at the skin interface, $\ln a_{w,x=L}$ is the natural logarithm of the water activity at the muscle interface and $\ln a_{w,s,\infty}$ is the natural logarithm of the water activity at the skin interface. As it was the case with the muscle interface, under dry salting the flux can only occur from the inside towards the outside of the herring fillet. This restriction is expressed as:

$$D_{a_w} \cdot C_{w,x=L} \left.\frac{\partial \ln a_w}{\partial x}\right|_{x=L} = 0, when\ \ln a_{w,s,\infty} = \ln a_{w,x=L} \tag{15}$$

At t=0, the water is heterogeneously distributed throughout the herring fillet:

241 $C_{w,0} = f(x)$, for t=0, x ∈ [0,L]

242 The mention non-ideal for the water transport properties means that the activity and not the
243 concentration gradient is the driving force

244

245 **4. Results and discussion**

246 *4.1 Steady state concentrations and equilibrium*

247 Table 1 displays the steady state concentrations and activities of salt and water, or in other words, the
248 interface concentrations that determine the steady state of the system. The steady state concentrations
249 were observed experimentally in the muscle and skin interfaces after 48h of contact and used to
250 compute theequilibrium salt and twater activities from equation 1 and 3, respectively. The steady state
251 salt concentrations were found to be highest for the muscle interface compared to the skin interface.
252 This also applied for the concentration of water. The steady state salt concentration ranged between
253 0.14-0.21 kg · kg^{-1} for the muscle interface and between 0.11-0.18 kg · kg^{-1} for the skin interface. In
254 respect to the water concentration, the steady state concentration for the muscle interface ranged
255 between 0.60-0.72 kg · kg^{-1} and between 0.42-0.66 kg · kg^{-1} for the skin interface (table 1).

256 *4.2 Concentration distributions*

257 *4.2.1 Salt*

258 Figures 2a, 3a and 4a show the distributions of salt at different times for 26 % and 16 % brining and
259 dry salting, respectively. The salt behaviour shows similar pattern for the different salting conditions
260 despite of the different steady state concentrations that are attained for each of them. The scatter in
261 the experimental salt and water concentration values may be due to the general biological variation
262 between individual herrings, in which induces the differences in the initial moisture content stated in

§2.4 (Nielsen, Hyldig, Nielsen, & Nielsen, 2005; Rodger et al., 1984). The shape of the salt distributions is at any time asymmetrical around x/L = 0.5, with a minimum around x/L = 1 that is especially noticeable at the beginning of the salting process. This suggests that either salt penetrates exclusively through the muscle interface located at x/L = 0, or that the flux through the skin boundary located at x/L = 1 is negligible. Some authors have found that salt diffusion from the skin side was inhibited by the skin as well as the layer of fat beneath the skin (Rodger et al., 1984). Similar observations were found in salmon under similar salting conditions (Gallart-Jornet et al., 2007), where the authors proposed that the subcutaneous fat layer in salmon represented an effective barrier for salt transfer.

The muscle interface does not reach the steady state concentration immediately after getting in contact with the salting environment. During brining in 26 % salt, the concentration in the region closest to the muscle interface (measured experimentally between x/L=0.05 and 0.13) has already reached 66 % of the steady state concentration after 1h 84 % after 6 h and 100 % after 48 h (Figure 2a, scatter plot). According to equation 3, and the water concentration values read from figure 2b (scatter plot) these translate into salt thermodynamic activities of 0.3 (1h), 0.49 (6h) and 0.61 (48h), over an equilibrium value of 0.61. Brining in 16% salt, the region close to the muscle interface (measured experimentally between x/L=0.03 and 0.12) reached 72 % of its steady state concentration after 6 h and around 90 % after 24 h (Figure 3a, scatter). Together with the water concentration values read from figure 3b (scatter), these translate into salt thermodynamic activities of 0.27 (6h) and 0.35 (24h), over an equilibrium value of 0.41. Finally, during dry salting the concentration in the region close to the muscle interface (measured experimentally between x/L=0.03 and 0.08) has reached 67 % of the steady state concentration after 6 h, and 92 % after 24 h (Figure 4a, scatter) which, together with the water concentrations read from figure 4b (scatter), correspond to salt activities of 0.5 (6h) and 0.85 (24h), over an equilibrium value of 0.99. As it can be seen in figure 5, the salt activity after 24 h is

287 already high and quite homogeneous throughout the samples, regardless of the salting conditions.
288 This suggests that the steady state is achieved shortly after 24 h of salting time. .

289 *4.2.2 Water*

290 Figures 2b, 3b and 4b show the distributions of water at different times for 26 % and 16 % brining
291 and dry salting, respectively. Water does not show the same transport behaviour as salt does. First,
292 because the initial water distribution is not homogenous, second, because of the seasonal variation in
293 herrings, the water content show a much larger variation than the salt content (Laub-Ekgreen,
294 Martinez-Lopez, Frosch, & Jessen, 2018). Finally, since the driving force is the water activity gradient
295 and not the concentration gradient as was the case for salt, the transport behaviour during dry salting
296 may significantly differ from the brining. In dry salting there is no water readily available in the
297 salting environment, water can only cross the interface towards the outside, while during brining,
298 depending on the water activities attained at any time at both sides of the boundaries, flux may occur
299 in both directions.

300 During dry salting, the water distribution seems to be somehow flat, with the muscle interface going
301 from 0.62 kg · kg^{-1} at 6 h to 0.60 kg · kg^{-1} at 24 h, and the skin interface going from 0.65 kg · kg^{-1} at 6
302 h, to 0.52 kg · kg^{-1} after 24 h (Figure 4b).

303 In the beginning of brining (6 h), the water distribution may show some curvature, either sinusoidal
304 (Figure 2b) or decreasing exponential-like (Figure 3b), which flattens progressively until it becomes
305 an asymmetrical straight line (Figure 2b) at the steady state (48 h). In general, lower water
306 concentrations were observed for fillets that were dry salted or brined in 26 % salt compared to the
307 brining in 16 % salt. This can be explained by the occurrence of protein denaturation and a decrease
308 in water holding capacity caused by the salt intake allowing water to leave the system (Boudhrioua

et al., 2009; Gallart-Jornet et al., 2007). As it can be seen in figure 3b, the shape of the concentration profile has not changed considerably with respect to the initial state after 6h of brining at 16%.

4.2.3 Water activity

Figures 6a, b and c show the water activity profiles at different times for dry salting, 26 % and 16 % brine, respectively, which is dictated by the salt and water distribution profiles. The transfer of salt through the muscle interface initiates the counter diffusion of water to equilibrate the activity gradient created by the sudden increase in salt concentration. Because of this, the water activity in the muscle interface decreased and over time the water activity profiles flatten until the whole fillet reached the steady state values displayed in table 1. As it can be seen in figure 6, after 24 h the water activity profile has already become homogeneous around the equilibrium value for dry salting (figure 6a) and 16% brine (figure 6c), and it is very close for the 26% brine. Comparing the salt, salt activity, water and water activity profiles, when the system reaches the steady state around 24 h; the water activity is homogeneously distributed, while the salt and the water concentrations are not. This suggests that the hypothesis of the water activity gradient being the driving force of the water transport has been verified.

4.3 Model predictions

Table 2 shows the root mean square error (RMSE) between the predictions of the model and the experimental datasets used for the parameter determination and validation. The data set marked with the letter a and b were used for determination of the mass transfer and diffusion coefficient of salt, respectively. Data sets marked with the letter c and d were used for determination of mass transfer and diffusion coefficient of water, respectively. The remaining data sets were used for model validation. In general, the model performs reasonably well. The RMSE for the prediction of the

distributions is below 0.03 kg · kg^{-1} for the salt, below 0.05 kg · $kg^{-1}$for the water, and below 0.042 for the water activity. Nevertheless, the predictions of the water concentration profiles are slightly worse at the beginning of the salting process. Besides the unavoidable experimental data dispersion, the main reason behind this can be explained by the model not taking into account the expansion or swelling of the tissue, as well as the use of the concentration gradient, together with a constant diffusion coefficient to describe the salt transport. This is discussed in the following sections

4.4 Influence of swelling/expansion.

The description of the system, as described in 3.2 and 3.3 considers the thickness of the system as invariable with time. Nevertheless, this is a simplification, since it is known that salt induces shrinkage and expansion of the muscle tissue. According to (Aliño, Grau, Fernandez-Sanchez, Arnold, & Barat, 2010), the muscle swells with an increasing NaCl concentration until the latter reaches 1M in the water phase (equivalent around 4.2 kg kg^{-1} if expressed in mass fraction, as used throughout this study), after which it shrinks. As it has been stated, although the salt enters the herring exclusively through the muscle interface, the water can leave the system through both the muscle and the skin interface. Consequently, the salt concentration corresponding to the maximum expansion will be locally reached at different times, thus becoming a potentially significant source of error.

As it can be deduced from figures 2a, 3a and 4a, at the initial stages of the kinetics, some regions of the herring are still at concentrations around 4.2 kg kg^{-1}: around x/L = 0.5 after 1h brining at 26% (figure 2a), between x/L = 0.75 and x/L = 1 after 6h brining at 16%, and the regions close to the skin interface after 6h of dry salting. The poorer predictability of the water distribution at the initial stages of the kinetics (figures 2b, 3b and 4b), could be mitigated by including an expansion/swelling mechanism in function of the local concentration of salt in the water phase of the herring.

4.5 Physical meaning of the coefficients

355 Table 3 displays the transport properties found for salt and water for the different salting conditions.
356 It was necessary to determine a coefficient set for each of the salting conditions in order to achieve
357 reasonable predictions of the salt and water concentration profiles. Nevertheless, some of the
358 coefficients are similar, which is the case for the salt diffusion coefficient for dry salting and 26 %
359 brining. The salt diffusion behaviour are almost identical for these two salting conditions. Since 26
360 % is actually a saturated salt solution, it can be expected that the diffusion behaviour of salt is very
361 similar for both situations. Contrarily, the diffusion coefficient for the 16 % brine is lower, which
362 appears somehow unexpected.

363 With respect to salt diffusion coefficients in herring, Rodger et al. (1984) found a value of 1.14×10^{-10}
364 m^2/s for Baltic herrings, which is approximately ten times smaller than what was found during this
365 work. A difference of around one order of magnitude can be expected when comparing diffusion
366 coefficients determined by a global method and a local method, as it is the case in this study
367 (Martinez-Lopez, Chalier, Guillard, Gontard, & Peyron, 2014). Vestergaard et al., (2007) determined
368 diffusion coefficient in meat by local concentration profiles and obtained an average salt diffusion
369 coefficient of 6.4×10^{-10} m^2/s, which is similar to the coefficient obtained for 16 % brine in this study.
370 Some authors have shown that the diffusion coefficient of salt in an aqueous solution is inversely
371 proportional to its molality (Vitagliano & Lyons, 1956). Although the hindrance of the interlinked
372 muscle network may result on generally slower diffusion than in a salt solution, a similar dependence
373 between the diffusion coefficient and the salt molality would have been expected (Wang, Correia,
374 and Tang, 1998).

375 For sardine fillets diffusion coefficients for moisture transfer have been found in the range 9.80×10^{-10}
376 to 1.20×10^{-8} m^2/s for dry salting conditions (Boudhrioua et al., 2009), which is within the same
377 range found in this study.

The salt mass transfer coefficient is lower for the dry salting, than for the brining. The mass transfer coefficients for the two brining conditions were identical. Since the salt crystals should undergo an additional step of solubilization in the water phase, in order to penetrate through the muscle interface, which is not necessary during brining, the magnitude difference between both coefficients fits with their expected physical meaning. The magnitude difference between the non-ideal mass transfer coefficients of both interfaces for water is such, that the flux through the skin interface is less significant than through the muscle interface. This appears more pronounced during dry salting and brining at 16 %.

4.6 Relationship between constant transport properties and driving forces

Figure 4, displays the experimental data for dry salting after 6 h with the coefficients found in table 3, as well as with another set of coefficients re-adjusted by hand for that dataset ($k_{s,m}$ = 5 ×10^{-7} m/s, D_s = 1.6 ×10^{-9} m/s and D_{aw} =1.9×10^{-9} m/s), which correspond to a 63 and a 126% of the $k_{s,m}$ and D_s displayed in table 3. As it can be seen, the RMSE for the salt varies from 0.0154 to 0.0168 kg·kg^{-1}, or 110 %, while the RMSE for water decreases from 0.0462 to 0.0268 kg·kg^{-1} or 58%. This way, a minimal variation in the salt transport properties does not significantly affect the predictability of the salt concentration distribution (Figure 4a), but does affect the predictability of the water (Figure 4b), thus potentially needing a re-adjustment of its non-ideal diffusion coefficient in order to obtain an optimal prediction.

This poorer predictability of the water distribution at the beginning of the kinetics is connected to the previous discussion about the absence of a clear trend between some transport properties and the salting conditions. These obey to i) the transport properties have been considered constant through the whole process, which in the practice requires a different coefficient dataset for each of the salting conditions; and ii) the concentration gradient has been considered to be the force driving the salt

transport. These two reasons are linked and can be formulated into a single one: the salt flux is inversely proportional to the molality; or in other words, as the water phase of the herring becomes saturated with salt, the salt transport speed decreases.

As it can be seen in equation 11, the activity gradient couples the water flux to the salt flux. This means that a decrease in the salt flux has an impact on the water flux, and consequently, on the predictabilities of both the salt and the water. According to equation 6, the force driving the salt transport is its concentration gradient, which means that a change in the water flux does not have any impact on the salt flux. Hence, since at the beginning of the kinetics, the salt concentration inside the muscle is at its lowest, the salt flux is at its maximum, and thus, the error on the predictability of the water is at its maximum.

5. Conclusion

In this work, the mathematical model of coupled salt and water transfer was validated for dry salting and brining of herring fillets. The model can predict salt and water concentration profiles and was developed for herring but is also valid for other types of muscle salting. The models is based on mechanistic principles that also help to understand why the system behaves the way it does. The experimental results confirmed that either the salt uptake occurs solely through the open muscle interface, or that the salt flux through the skin interface was so low that it was negligible.

Despite the use of the salt concentration gradient and not the salt activity as driving force, and the assumption of a constant salt diffusion coefficient, the predictions of the salt concentration profiles and water activity are reasonably good. Nevertheless, this affects the predictability of the water concentration profiles in the initial stages of the kinetics. The next step would be to develop the numerical method, so either the salt activity gradient or the water phase salt concentration gradient can be used as driving force instead of the concentration gradient. This would allow the use of

constant transport properties for the salt, on the same basis as it is done for the water. In any case, the model of salt and water transfer is a useful tool to improve the herring salting process and to study the effect of different processing parameters.

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Author contributions

M.H. Laub-Ekgreen developed the methodology that allowed to measure the salt and water concentration profiles, gathered the experimental data and wrote the paper. B. Martínez-López conceived the paper, adapted the model of its own conception to the particularities of herring and determined the parameters and revised critically the manuscript. F. Jessen provided insight about herring and revised the manuscript critically.

List of symbols

$C_{s,0}$	Initial concentration of salt in wet basis in kg salt/(kg salt +kg water + dry matter) and now referred to as kg·kg^{-1}	(kg·kg^{-1})
$C_{s,m,\infty}$	Solubility limit of salt in the herring muscle	(kg·kg^{-1})
$C_{s,s,\infty}$	Solubility limit of salt in the herring skin	(kg·kg^{-1})
$C_{w,0}$	Initial concentration of water in wet basis in kg water/(kg salt +kg water + dry matter) and now referred to as kg·kg^{-1}	(kg·kg^{-1})
m	Molality (mol salt/kg water)	(mol·kg^{-1})
$C_{w,m,\infty}$	Solubility limit of water in the herring muscle	(kg·kg^{-1})
$C_{w,s,\infty}$	Solubility limit of water in the herring skin	(kg·kg^{-1})

D_s	Salt diffusion coefficient	(m^2/s)
D_{aw}	Non-ideal water diffusion coefficient	(m^2/s)
$k_{s,m}$	Salt mass transfer coefficient, muscle interface	(m/s)
$k_{s,s}$	Salt mass transfer coefficient, skin interface	(m/s)
$k_{aw,m}$	Non-ideal water mass transfer coefficient, muscle interface	(m/s)
$k_{aw,s}$	Non-ideal water mass transfer coefficient, skin interface	(m/s)
$a_{w,m,\infty}$	Water activity limit, muscle interface	(-)
$a_{w,s,\infty}$	Water activity limit, skin interface	(-)

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Tables:

Table 1: Steady state concentrations and equilibrium activities of salt and water in the muscle and skin interface and brine.

	Dry salting	**Brine 26 %**	**Brine 16 %**
	C_s (kg·kg^{-1})		
Brine	-	0.26	0.16
Muscle	0.21	0.17	0.14
Skin	0.18	0.11	0.13
	C_w (kg·kg^{-1})		
Brine	-	0.74	0.84
Muscle	0.6	0.65	0.72
Skin	0.52	0.42	0.66
	a_s		
Brine	-	1	0.41
Muscle	0.99	0.61	0.41
Skin	0.99	0.61	0.41
	a_w		
Brine	-	0.76	0.89
Muscle	0.76	0.82	0.88
Skin	0.76	0.82	0.88

Table 2: RMSE values

		RMSE		
Salting condition	**Time (h)**	C_s (kg·kg^{-1})	C_w (kg·kg^{-1})	a_w -
Dry salting	6	0.0154 **a,b**	0.0462 **c**	0.0126
	24	0.0263	0.0167 **d**	0.0359
	48	0.0286	0.0161	0.0414
26% brine	1	0.0137	0.0564	0.0144
	6	0.0153 **a,b**	0.0321 **c,d**	0.0194
	24	0.0155	0.0295	0.0180
	48	0.0114	0.0365	0.0195
16% brine	6	0.0265	0.0490 **c,d**	0.0197
	24	0.0183 **b**	0.0324	0.0186
	48	0.0226	0.0146	0.0241

a: used for the determination the mass transfer coefficient of salt,

b: used for the determination of the diffusion coefficient of salt,

c: used for the determination of the mass transfer coefficients of water and

d: used to for the determination of the diffusion coefficient of water.

Table 3: Transport properties of salt and water in fish at 2 °C

Salting condition at 2 °C	D_s ($\times 10^{-10}$) (m^2/s)	D_{a_w} ($\times 10^{-10}$) (m^2/s)	$k_{s,m}$ ($\times 10^{-7}$) (m/s)	$k_{aw,m}$ ($\times 10^{-7}$) (m/s)	$k_{aw,s}$ ($\times 10^{-10}$) (m/s)
Dry salt	12.6	31.6	7.94	3.98	1.25
26% brine	15.8	25.1	19.9	19.9	794
16% brine	7.94	15.8	19.9	3.16	0.013

Figures

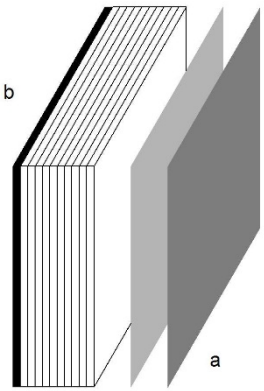


Figure 1: Illustration of the slicing procedure of the fish sample to measure the local concentrations of salt and water, a) is the surface of the fillet, i.e. slice no. 1 and b) is the skin side. Salt and water is measured on each of the slices.

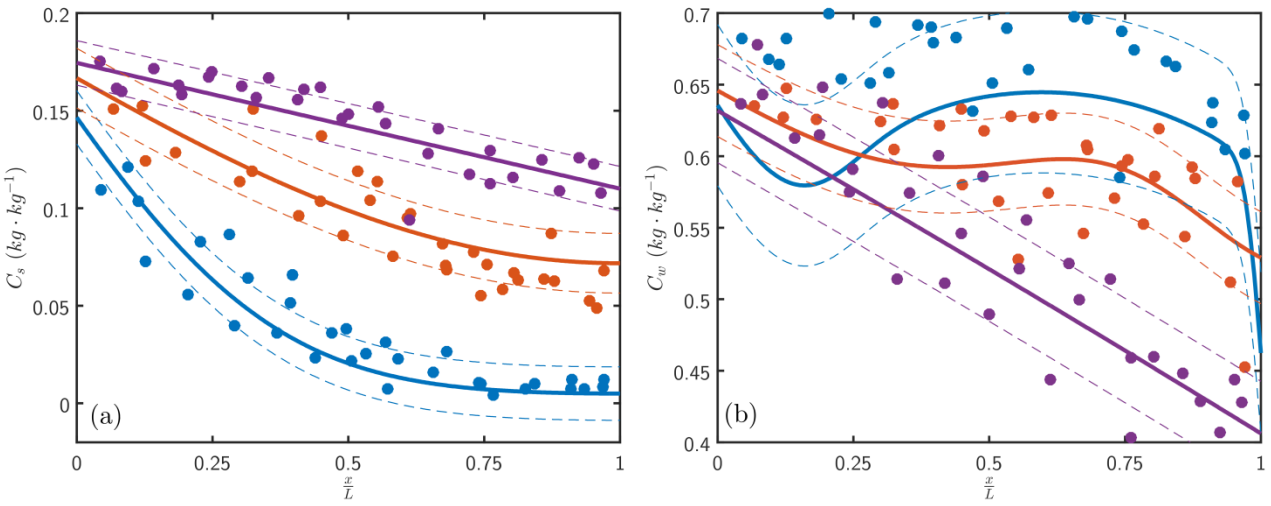
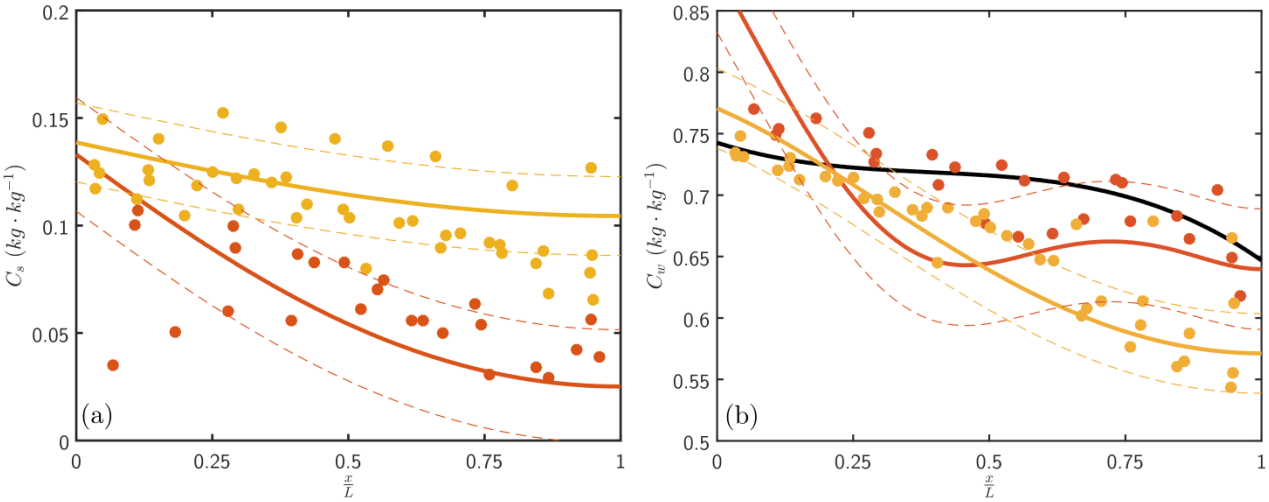


Figure 2: Concentration profiles ($kg \cdot kg^{-1}$) of salt (a) and water (b) after salting in 26% brine: 1 h (blue), 6 h (red) and 48 h (purple) and water ($kg \cdot kg^{-1}$) for 1h (blue), 6 h (red) and 48 h (purple) (b), (-) model fit, (---) RMSE. x/L= 0 is the muscle interface and x/L=1 is the skin interface.



583

584 Figure 3: Concentration profiles of salt (kg·kg^{-1}) (a) and water (kg·kg^{-1}) (b) for herring fillets salted in 16 %
585 brine for 6h (red) and 24 h (yellow), (-) model fit, (---) RMSE. x/L= 0 is the muscle interface and x/L=1 is
586 the skin interface. The water plot (b) includes the smoothed average initial concentration of water,
587 represented as a continuous black line.

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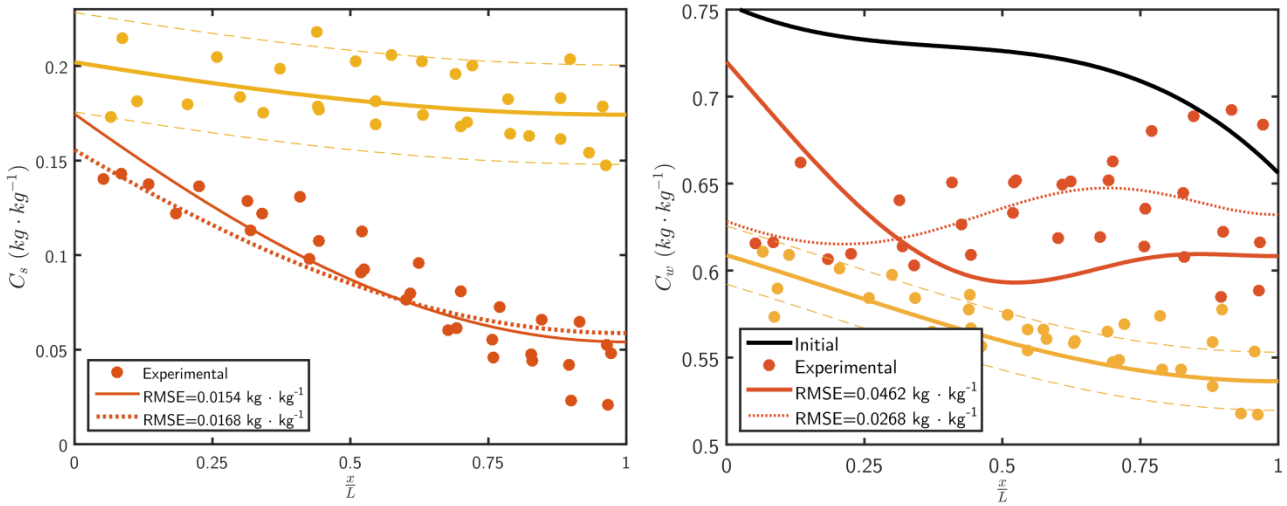

Figure 4: Concentration profiles for salt (a) and water (b) after dry salting for 6 h (red) and 24h (yellow). The dots represent the experimental data, the continuous line, the predictions of the model by using the parameters from table 3, dashed line the RMSE, while the dotted line has been made with $k_{s,m}$ = 5 ×10^{-7} m/s, D_s = 1.6 ×10^{-9} m^2/s and D_{aw} =1.9×10^{-9} m^2/s. The water plot (b) includes the smoothed average initial concentration of water, represented as a continuous black line.

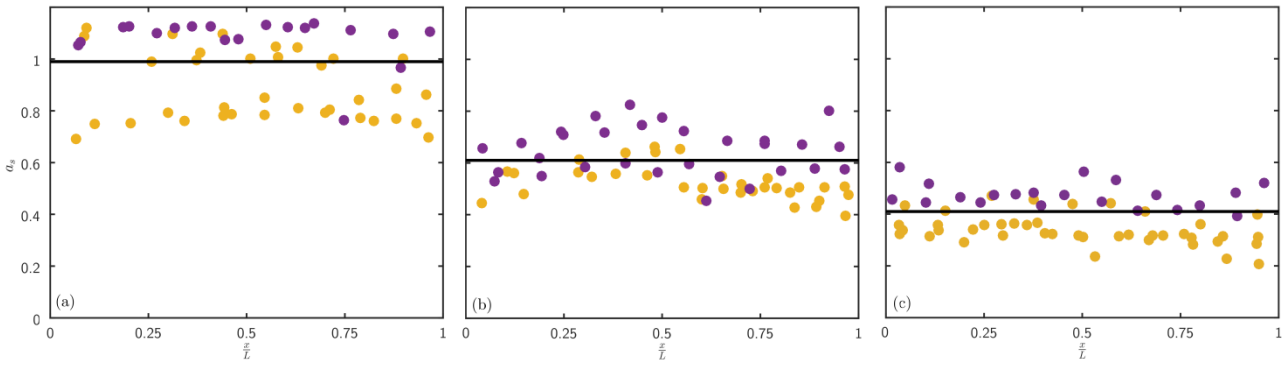

Figure 5: experimental salt activities in function of the dimensionless position at 24h (yellow) and 48h (purple) for dry salting (a), 26% brine (b) and 16% brine (c). The continuous black line represents the equilibrium values, that can also be found in table 1.

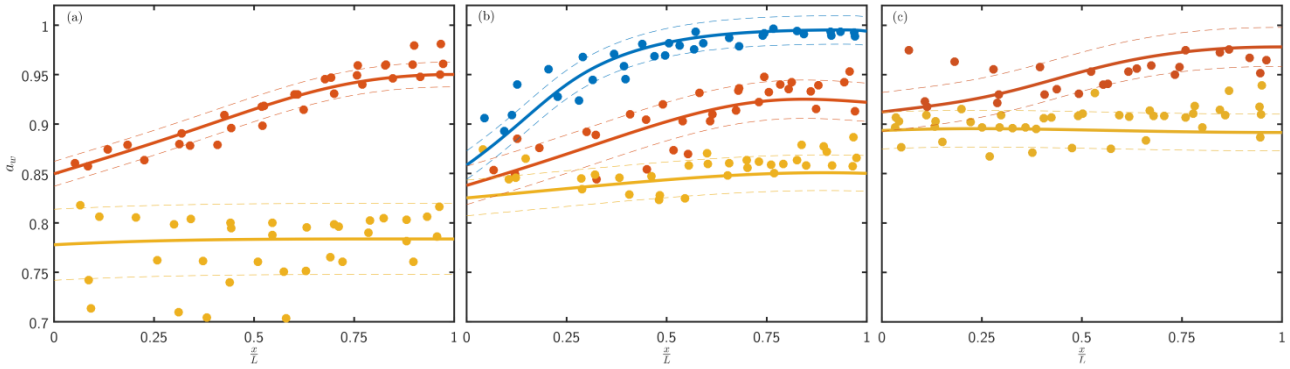

Figure 6: Water activity profiles for herring fillets after dry salting for 6 h (red) and 24 h (yellow) (a), brining in 26 % salt for 1 h (blue), 6 h (red) and 48 h (purple) (b) and brining in 16 % salt for 6 h (red) and 24 h (yellow) (c). (-) model fit, (---) RMSE and (x/L= 0 is the muscle interface and x/L=1 is the skin interface).

Paper III

Non-destructive measurement of salt using NIR spectroscopy in the herring marinating process.

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Non-destructive measurement of salt using NIR spectroscopy in the herring marinating process

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ABSTRACT

The salt content is one of the most important quality and safety parameters in the manufacturing process of marinated herring, which needs to be controlled during processing. Standard methods are often destructive and time consuming, and therefore a nondestructive and fast method is needed. Near-infrared (NIR) spectroscopy was measured on marinade samples from the herring marinating process in order to investigate the potential of NIR as a fast method to determine the salt content in marinade and in fish. The spectral region 1100–1300 nm had the highest positive correlation with the measured salt values. A principal component analysis performed on the NIR spectra showed that the first principal component described the evolution of the spectra according to the determined salt values. A partial least-squares regression model between the selected region of the NIR spectra and the salt content of the fish gave a correlation coefficient of 0.81 and a prediction error (RMSECV) of 0.41 g/100 g with the prerequisite that salt concentration in fish and marinade was in equilibrium. The results indicate that NIR spectroscopy can be used as a fast and non-destructive method for assessing the salt concentration in fish during the herring marinating process in order to ensure product safety.

1. Introduction

Marinated herring products are traditionally consumed in Northern European countries and manufactured by a process using a solution of sodium chloride and acetic acid in order to increase the ionic strength and decrease pH and hereby preserving the fish making it available for consumption most of the year (Rodger, Hastings, Cryne, & Bailey, 1984). This process is based on passed down experience and years of traditions and often consists of an intermediate salt brining followed by the marinating process using a solution of salt and acetic acid. Marinated herring products are semi manufactured products with no prior freezing step and no sequential heat treatment. Salt is one of the key preservatives for these herring products, but is also an important factor for the sensory characteristics (quality) of the product. One of the main safety issues is the presence and viability of the *Anisakis* larvae. A study showed that the mortality of the *Anisakis* larvae was mostly influenced by the salt concentration in the muscle liquid phase compared to the concentration of acetic acid and an adequate salt content in the fish liquid phase is important in order to achieve a safe product (Karl, Roepstorff, Huss, & Bloemsma, 1995). Salt is an important quality and safety parameter, which needs to be determined and controlled during processing.

Quality control is typically conducted at the end of the herring marinating process, where samples of fish are collected for analysis and visual evaluation. A common method for salt analysis involves an aqueous extraction of salt from the sample and titration with standardized silver nitrate (AOAC 976.18). This method is accurate, but also destructive and time consuming and difficult to run in a production setting. In some productions the final quality control is conducted using near-infrared (NIR) spectroscopy, where samples of fish are collected for analysis, however, preparing the samples for analysis is destructive and can be time consuming as well. Besides, it is known that variability between herring fillets occurs, especially in the fat content (Aidos, van der Padt, Luten, & Boom, 2002; Lane, Westgate, & Koopman, 2011; Nielsen, Hyldig, Nielsen, & Nielsen, 2005), and sampling of some fillets may not be appropriate to characterize the whole batch. Sampling of the surrounding brine is very attractive and may be more representative and indeed more accessible than sampling the whole fish during processing. In this way the concentration of the marinade, when in equilibrium with the herring muscle, can be used as a quality parameter throughout the entire herring marinating process.

Non-destructive and rapid methods for salt detection are promising

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and studies show that NIR spectroscopy was used to determine the salt content in aqueous solutions (Hirschfeld, 1985; Lin & Brown, 1993), in meat (Begley, Lanza, Norris, & Hruschka, 1984), in cod (Galvis-Sanchez, Tóth, Portela, Delgadillo, & Rangel, 2011), in cured salmon roe (Huang, 2001) and in hot smoked salmon (Lin, Cavinato, Huang, & Rasco, 2003).

NIR spectroscopy is a useful analytical technique for biological samples and works by measuring the amount of light, which is absorbed by the sample as a function of the wavelength (Galvis-Sanchez et al., 2011). The method is based on vibrational modes of molecules mainly C-H, O-H, and N-H functional groups, which can be observed as overtones and combinations in the NIR spectrum (Huang, 2001; Svensson, Nielsen, & Bro, 2004). While, sodium chloride (NaCl) has no specific absorption band(s) in the NIR region, it is known that salt in solution causes changes in the height, width and position of the absorbance bands of water (Hirschfeld, 1985; Lin & Brown, 1993). The bands become narrower and shift to a shorter wavelength with increasing NaCl concentration compared with the bands of pure water (Lin & Brown, 1992). For that reason, it is expected to find information about the changes in salt concentration in the marinade over time in the herring marinating process. NIR is a good choice for quality control, not only can it be used to determine the salt content during the manufacturing of marinated herring, but also simultaneously determine other parameters such as protein, sugar and fat content (Begley et al., 1984). Despite being a promising technique there are also some drawbacks to consider, e.g. water is the major constituent of herring marinade, which strongly characterize the spectral information in NIR with peaks around 1450 nm and 1886 nm.

The marinade is a heterogeneous medium consisting of fat, protein and water, which all absorbs in the NIR region resulting in overlapping signals (Grassi, Amigo, Lyndgaard, Foschino, & Casiraghi, 2014). For that reason, the use of chemometrics is needed in order to extract the relevant information from the NIR data. The objective of the present study was to investigate if NIR spectroscopy could be used to determine the salt content in fish water phase in marinated herring fillets by obtained spectra of marinade. Multivariate data analysis and prediction modelling between NIR spectroscopy and salt concentration in marinade and in fish were used in order to study the relation between the NIR measurements and the actual salt concentration.

2. Materials and methods

2.1. Experimental data

The effect of the increasing salt concentration in the spectra was studied by obtaining NIR spectra of 0, 13, 16 and 26 g/100 g NaCl. The main experiment intends to mimic the industrial marinating process of herring fillets, and consists on brining followed by marinating. The brining was performed using different concentrations and times (Fig. 1), while the marinating was carried out in a solution of 6.7 g/100 g of acetic acid and 5 g/100 g salt during 35 days at 2°C. A different container was used for each of the stages and each of the batches.

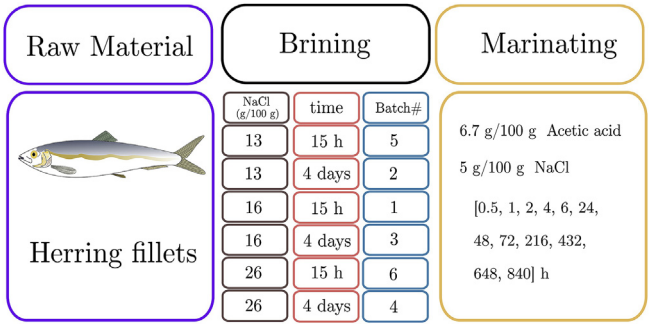

Fig. 1. The experimental set-up for the herring marinating experiment.

An overview of the experiments is shown in Fig. 1.

At each sampling time herring fillets were drained for 1–2 min using a sieve and two individual brine samples (app. 15–25 ml brine/marinade) and three fillets were taken from each bucket. Upon analysis, the brine was centrifuged at 3800 g for 20 min at 5°C to remove tissue part and insoluble matter and brine and fish samples were kept at −40°C until analyses were carried out. Herring fillets were rinsed under running water in order to avoid excess salt crystals on the flesh surface before chemical analyses were carried out. The sampling times were 0.5,1, 2, 3, 4, 5, 6, 24, 48, 72, 216, 432, 648 and 840 h giving 28 brine samples and 42 fillets representing each batch.

2.2. Salt and moisture content

The salt content of brine, marinade and fish (flesh and skin was minced) was determined by titration with $AgNO_3$ (Titrator, 785 DMP Titrino with a magnetic stirrer, Metrohm) in accordance to AOAC methods (AOAC 976.18 in combination with 937.07) (AOAC, 2000a, 2000b). The dry matter content of fish samples was determined after heating the sample at 105 °C for 48 h where a stable sample weight was achieved. The salt and dry matter content were measured on different samples from the same fillet.

2.3. Near-infrared spectroscopy

NIR spectra of brine/marinade were measured with a Fourier transform spectrometer (QFA-flex, Q-interline) using a cuvette with a light path length of 8 mm in transmission mode. Each sample was measured with the average of 128 scans (total duration approximately 40 s) over the spectral range of 1000–2500 nm (10.000–4.000 cm^{-1}) with a spectral resolution of 16 cm^{-1}. All samples were brought to room temperature by placing the brine samples in a water bath at 21°C for 30 min and in room temperature for 30 min before measuring and then the samples were measured over the course of two days. Air was used as the background for all spectra obtained and measured before the samples were measured each sampling day.

2.4. Data processing

Initial multivariate data analysis was performed by principal component analysis (PCA). The spectra were pre-processed using Standard Normal Variate (SNV) in order to minimise the effect of additive and multiplicative effect to the spectrum baseline (psychical effects due to the sample matrix) as well as noise and highlight modifications due to the chemical composition (Rinnan, Berg, & Engelsen, 2009).

Partial Least Squares regression (PLS) models were built, in order to relate the NIR spectra to the concentration of salt in brine/marinade based on the reference measurements from storage experiments. The spectra were pre-processed as described earlier. The pre-processed data as well as the variable to predict were mean centred before fitted with PLS models.

Cross-validation was conducted using Venetian blinds with 10 splits including 4 samples per split ensuring that replicates were kept together. The predictive performance was tested using the root mean squared error of cross validation (RMSECV) and the correlation coefficient (R^2) of the predicted value and the reference value. An average value of the salt content in the fish flesh (n = 3) is used in order to correlate the value to the salt concentration in brine/marinade. The RMSECV is given by comparing the predicted value and the reference values as shown in eq. (1).

$$RMSECV = \sqrt{\frac{\sum (y - \hat{y})^2}{n}} \quad (1)$$

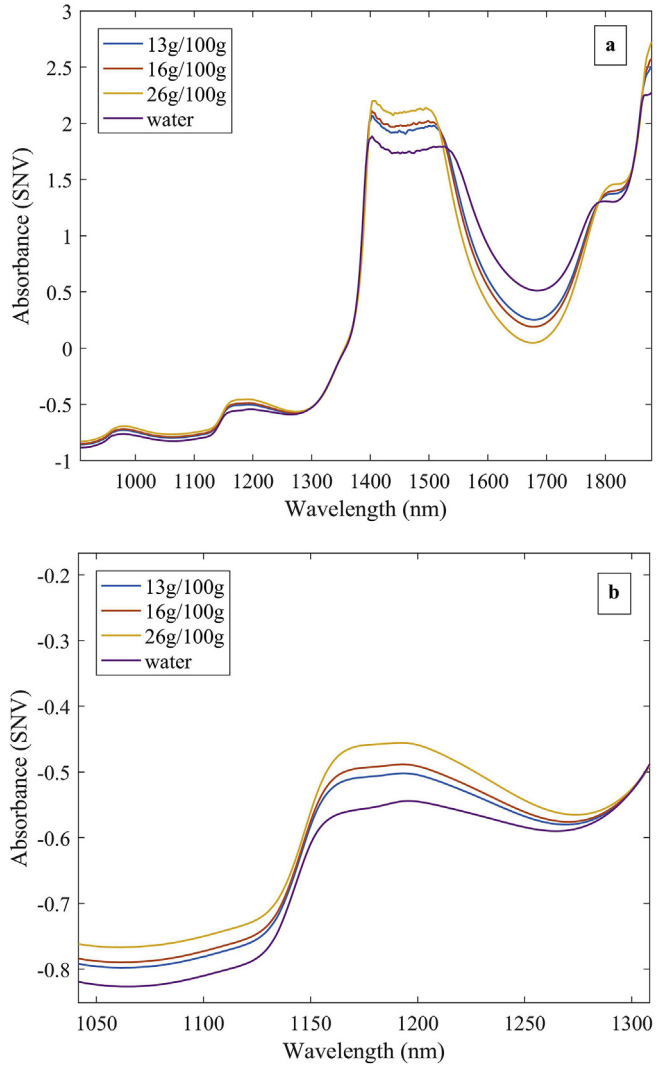

Fig. 2. NIR spectra (SNV) of salt solutions of 0, 13, 16 and 26 g/100 g NaCl for the region 900–1900 nm (a) and a zoom of the region 1150–1300 nm (b).

Where y and $\hat{y}$ represent the measured reference (salt g/100 g) and the predicted value (salt g/100 g), respectively, and n is the number of samples. The number of Latent variables (LVs) included in the models was evaluated by inspecting the root mean square error of cross-validation (RMSECV), selecting the number of LVs where the curve for RMSECV flattened out or had a minimum. Additionally, in order to find the uncertainty of the prediction error for each PLS components, an additional Monte Carlo cross validation was conducted by randomly dividing the dataset into a calibration and validation set and calculating the RMSEC, RMSECV and RMSEP 100 times.

All models were developed in the PLS Toolbox (Eigenvector Research Inc., Wenatchee, WA) working under MATLAB 2016a v. 8.1.1 (The MathWorks, Natick, MA USA).

3. Results and discussion

3.1. Influence of the increasing salt concentration on NIR spectra

Fig. 2 shows the NIR spectra (SNV) of four sodium chloride solutions varying between 0 and 26 g/100 g (w/v), which were used to study whether NIR spectroscopy could detect the salt in different concentrations. The four water absorbance bands were located around 980, 1200, 1450 and 1780 nm (Fig. 2). The main peak around 1450 nm is related to O-H first overtone of water (Grassi et al., 2014), and the light is heavily absorbed by water and here considered as noise. Weaker absorption was seen at 980, 1200 and 1780 nm. Dissolving different concentrations of NaCl in water resulted in changes in the wavelengths and intensity of the water bands. For the region 1600–1700 nm increasing salt concentration resulted in a decrease in absorbance intensity and the water bands became narrower and shifted to the shorter wavelength (Fig. 2a). Fig. 2b shows a linear increase in the water absorbance bands around 1200 nm with the increasing salt concentration. The changes in the water absorbance bands due to sodium chloride are probably related to the weakening or strengthening of the hydrogen bonding network (Lin & Brown, 1992), where chloride is thought to have the greatest effect (Begley et al., 1984).

Since water absorption is lower in the shorter wavelengths (Pedersen, 2002) and the change in salt concentration can be observed as a linear change in the water bands (around 1200 nm), it is believed that the change in salt concentration in the marinade is best observed in the shorter wavelength region. Thus, even though sodium chloride has no specific absorption bands in the NIR region it is possible to detect the change in salt concentration due to salts effect on the water absorbance bands (Huang, 2001), and these results provide a basis for the application to biological matrices.

3.2. Herring marinating study

Fig. 3 shows the concentration development of the herring water phase salt (WPS) and the marinade. As it can be seen, there is an abrupt drop in salt concentration of the herring WPS and a simultaneous and equally abrupt increase in the salt concentration of the marinade. This behavior was observed in all six batches, and can be explained by the higher salt concentration in the herring water phase after brining compared to the initial salt concentration of the marinade. This way, the transport occurs from the herring to the marinade, until the concentration in the water phase of the herring is equal to the salt concentration in the marinade (Birkeland, Sivertsvik, Neilsen, & Skåra, 2005). As a general trend, the salt concentration in the fish water phase was a little higher compared to the concentration of the marinade (Fig. 3). The salt concentration ranged from 4.4–9.2 g/100 g for marinade and 6.2–10.7 g/100 g in fish water phase. One batch of fish (E) did not reach equilibrium with the marinade and was discarded from the data set.

Fig. 4a shows the herring WPS concentration vs the salt concentration in the marinade for all the time points for which sampling is

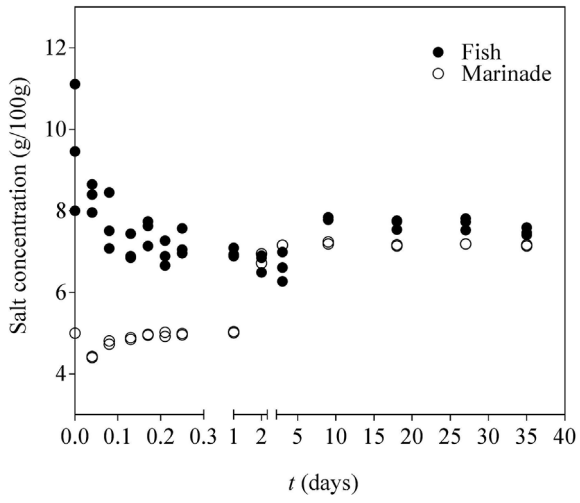

Fig. 3. Change in salt concentration of the brine and the fish water phase (WPS). Filled circles represents the salt concentration in the fish (water phase), and the empty circles represents the salt concentration of marinade during storage for one representative experimental batch.

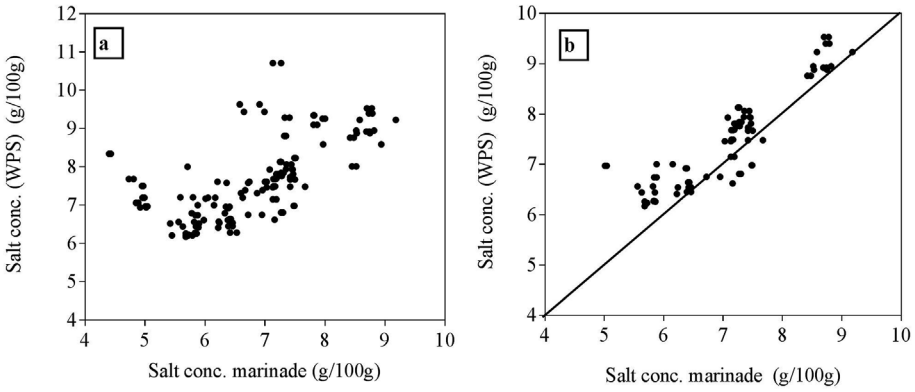

Fig. 4. Salt concentration in the marinade and the fish (WPS). Salt concentration of the marinade vs. the water phase salt (WPS) in the fish during the full storage time, 0.5–35 days, r = 0.63, n = 156 (a), and from 24 h to 35 days, r = 0.90, n = 84 (b), (-line) the target line for concentration equilibrium between the marinade and the fish muscle.

available. In comparison, Fig. 4b shows the same data, but only for the time points between 24 h and 35 days of marinating. A Pearson correlation coefficient was computed to assess the relationship between the salt concentration in marinade and fish for the full marinating time (0.5–35 days) and after 24 h of storage to 35 days, confirming the positive correlation between the two variables with r = 0.63 and r = 0.90, respectively. This higher correlation coefficient for the samples taken after 24 h was expected, since that is the time point after which the system can be considered in equilibrium, and gives an indication that the concentration in the marinade gives a reasonable concentration of the WPS in the fish, provided that the system is in equilibrium.

3.3. NIR spectroscopy

Fig. 5a shows the NIR spectra (SNV) collected from the marinade during the herring marinating process. The spectra were similar to the spectra collected from the salt solutions in the preliminary study with four main water absorbance bands. Light was heavily absorbed at 1450 nm (related to O-H first over tone) and considered as noise likewise in the preliminary salt study (Fig. 2). Fig. 5b shows in detail the spectral region 1150–1300 nm where the increase in salt concentration is well highlighted with the increase in intensity (absorbance). A sharp decent is seen in the region 1550–1650 nm, where the intensity decreased with increasing salt concentration (Fig. 5a) similarly to the preliminary salt study (Fig. 2). The correlation coefficients were calculated for each wavelength of the NIR spectra (SNV) against the chemical determined salt values of the marinade in order to find the region in the spectra that had the highest correlation (Fig. 5c). The region at approximately 1100–1300 nm gave the highest positive correlation with the measured salt values and the region around 1500–1800 nm gave the highest negative correlation. The average NIR spectra (SNV) were colored according to the correlation coefficients (Fig. 5d) in order to visualize the wavelengths containing most information about the salt concentration changes and that could be favorable to include in the calibration model. The spectral region 1170–1290 nm was selected for further analysis because of the high correlation to the actual salt concentration values hence contributing the most to the predictive performance and the reduced impact of water in this region (Pedersen, 2002).

3.4. Principal component analysis

Prior to regression analysis, an exploratory analysis of the spectral data was performed in order to gain an overview of the data and find possible clusters among the samples collected from the six batches of marinade. PCA was conducted on the selected region 1170–1290 nm because of the high correlation between this region and the actual salt values that was described in sections 3.1 and 3.3. Fig. 6a shows the score plot of the first and the second principal components, PC1 and PC2, respectively, with samples classified according to their batch number. As it can be seen, the spectra group reasonably well in function of their batch number. The differences between the six batches have two immediate explanations. The first and most evident one is the different (pre-brining) contact time and brine concentration used for each batch. This results in the batches brined in 26 g/100 g NaCl achieved a higher salt concentration compared to those brined in 13 g/100 g NaCl. The second one is the salt diffuse to the marinade from those fillets with a higher salt concentration at the beginning of the marinating (Fig. 3). Fig. 6b describes the evolution of the spectra according to the salt concentration of the marinade. Marinade samples collected from batch 2 and 5 with low salt concentration have negative PC1 values and are grouped together and samples collected from batch 1, 3 and 6 with higher salt concentration have positive PC1 values are also grouped together. Samples collected from batch 4 with the highest concentration of salt, have positive PC1 values and are separated from the others. Fig. 6c shows the loadings for the PCA model for PC1 and PC2 on the selected spectral region. The loading plot shows the relationship between the principal components and the important wavelenghts. The highest loading value for PC1 is found around 1200 nm, which is where a water absorbance band is located and is affected by increasing salt concentration.

3.5. Multivariate regression

Multivariate regression was performed in order to correlate the actual salt content of the marinade and herring to the pre-treated NIR spectra. The PLS models were evaluated in terms of root mean square error of calibration (RMSEC) and cross-validation (RMSECV). The main objective of this study was to investigate the ability of NIR spectroscopy to determine the salt concentration in herring muscle by the obtained spectra of marinade. For that reason initial PLS models were conducted for salt content in marinade in order to investigate how well the salt concentration could be predicted in the herring marinade. Comparing results from the model on the entire spectral range (without 1400–1550 nm due to full absorption of light) and the model including only the selected region, performed equally well (Table 1). This confirms that the removed wavelengths do not contribute in explaining the salt content of marinade. Additionally, this reduces the number of spectral variables, hence the model complexity, which ultimately results in a better stability of the calibration model (Ye, Gao, Li, Yuan, & Yue, 2016). For the sake of comparison, the effect of using exclusively samples after 24 h of marinating resulted in an improved model using 2 LV, R^2 was 0.91 and RMSECV was 0.27 g/100 g, which is illustrated in Fig. 7a. The same analysis was used to determine the salt content in the herring muscle water phase leading to a 5 factor model, R^2 was 0.81 and RMSECV is 0.41 g/100 g including samples after 24 h of marinating. As expected the predictability improves including samples after 24 h of marinating compared to the full marinating time (Table 1) because of the effect of equilibrium described in section 3.2.

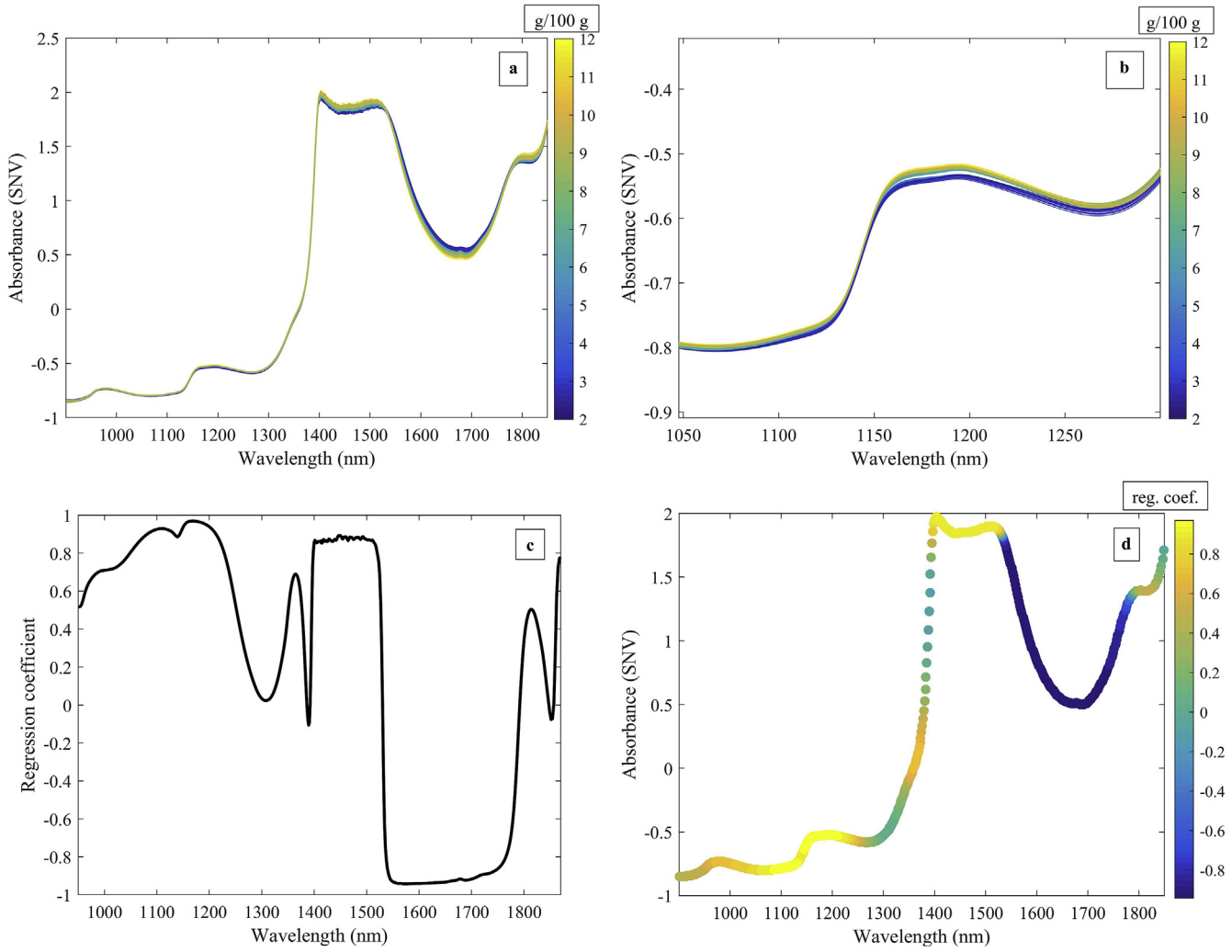

Fig. 5. NIR spectra (SNV) collected from the marinade in the region 900–1850 nm (a), and detail of the region 1150–1300 nm (b) both colored according to salt concentration (g/100 g). Correlation coefficients of the NIR spectra (SNV) and the measured salt concentration (g/100 g) (c) and the average NIR spectra (SNV) colored according to the calculated correlation coefficients (d).

The external validation procedure determines the predictive ability based on a sample set, which was not included in the model. In this study, the external validation was conducted using the validation set of 10 samples randomly selected (100 times) from each batch. In Fig. 8 the prediction error (RMSEC, RMSECV and RMSEP) is given as a function of the number of PLS components for the model (WPS in fish) based on the selected spectral region (1170–1290 nm). The optimum model rank is 5 for RMSEC, RMSECV and RMSEP, which is also in agreement with the results obtained of the PLS model of WPS illustrated in Fig. 7b. The prediction error at 5 LV is given 0.36 g/100 g ± 0.02 g/100 g, 0.40 g/100 g ± 0.02 g/100 g and 0.39 g/100 g ± 0.06 g/100 g for RMSEC, RMSECV and RMSEP, respectively, and these results indicate that the predictive ability of the model was good as the difference between RMSECV and RMSEP was small.

4. Conclusion

The prediction of the equilibrium concentration of NaCl in the marinade and the herring WPS is presented here to show the potential of NIR spectroscopy for fast and nondestructive determination of salt during the herring marinating process. Sampling of marinade is easily performed compared to sampling of the herring fillets. The preliminary salt study suggested that NIR spectroscopy in the range of 1170–1290 nm carry information related to the changes in salt concentration of sodium chloride solutions. A PCA and inspections of the NIR spectra also confirmed that the spectral region (1180–1290 nm) carried information associated with the change in salt of herring marinade. Calibration models were established for salt in the marinade and the fish muscle water phase independent on the different brining procedures applied to the six batches. NIR measurements are a good alternative to the timeconsuming sampling and chemical analysis of herring fillets in order to determine the salt content and have potential to be implemented in the herring marinating industry. Moreover, it opens up for new opportunities for faster measurements of the change in salt during processing and with the benefit of measuring several parameters simultaneously. These results contribute to the optimization of the process control in the herring marinating industry; however, further studies are needed. Lab models are a good first approach to study the feasibility of NIR for process control, however, building calibration models to be used in large scale industrial food processes should be conducted in the industrial setting. The samples chosen for the calibration should span the variability in both the process (such as storage time, temperature, humidity and raw material variability) and the target constituents (such as variation in salt and acetic acid concentration). Emphasis should be put on correct sampling to ensure that both the NIR measurements and the reference sampling (that is required for making calibration models) are conducted with a good representation of the entire batch.

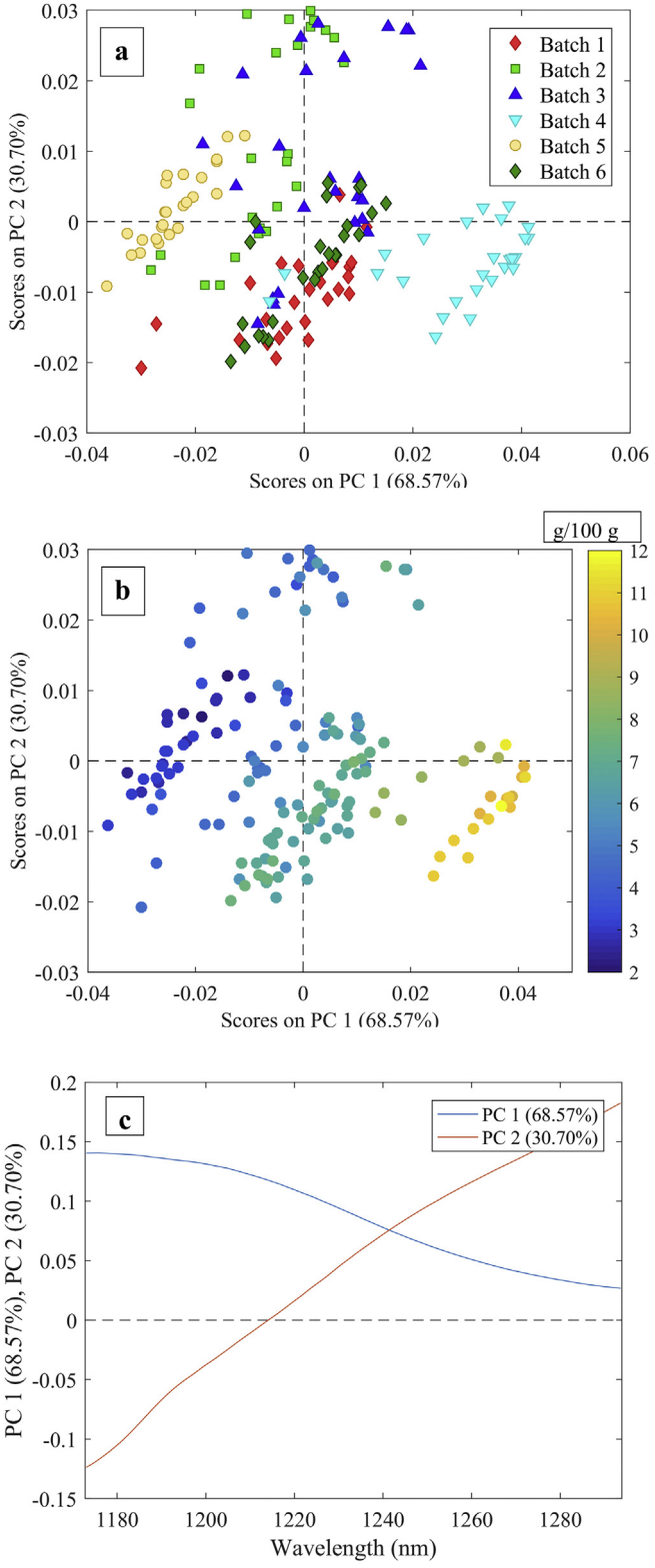

Fig. 6. Principal component analysis (PCA) of the NIR spectra collected. Score plot of PC2 vs. PC1 colored according to batch number (a) and PC2 vs. PC1 colored according to the measured salt concentration in the marinade (b) for the selected region 1170–1290 nm.

Table 1
Statistics of calibration models of salt concentration in marinade and fish samples.

Parameter	Spectral region (nm)	Calibration				
		n	RMSEC (g/100 g)	RMSECV (g/100 g)	R^2 (CV)	LV
Marinade	900–1400; 1550–1850	156[a]	0.29	0.30	0.88	4
Marinade	1170–1290	156[a]	0.29	0.30	0.88	4
Marinade	1170–1290	84[b]	0.26	0.27	0.91	2
WPS	1170–1290	156[a]	0.60	0.62	0.64	5
WPS	1170–1290	84[b]	0.35	0.41	0.81	5

WPS: fish water phase salt (g/100 g), *RMSEC*: root mean square error of calibration, *RMSECV*: root mean square error of cross validation, R^2: determination coefficient for calibration set, a: all samples included (0.5 h–35 days), b: samples after equilibrium included (1–35 days).

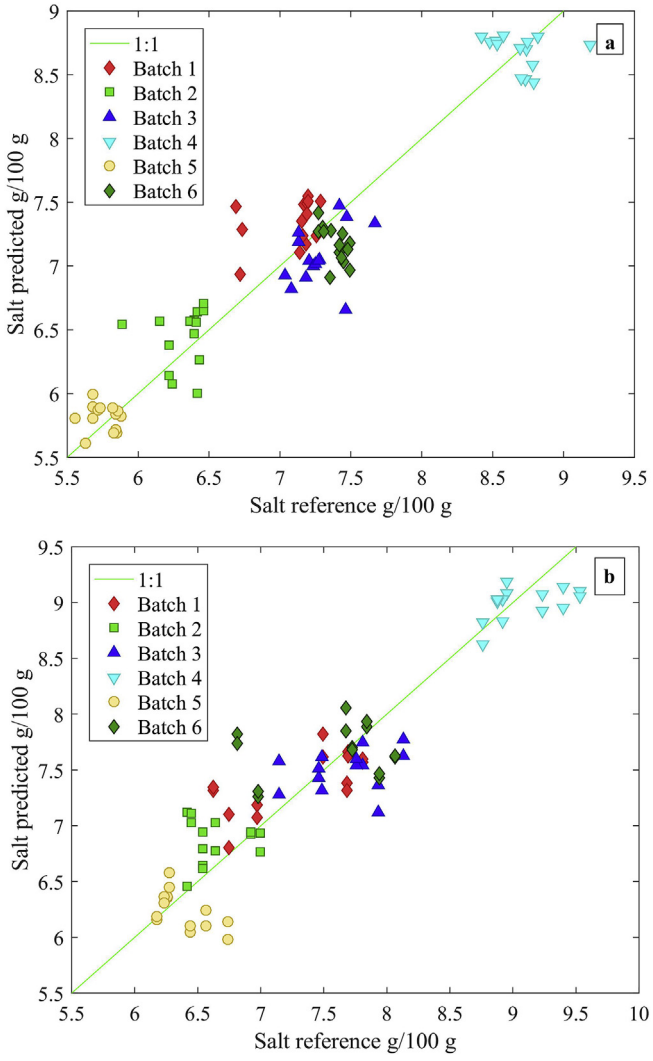

Fig. 7. Predicted vs. measured salt concentration in marinade (g/100 g) (a) predicted vs. measured salt in fish water phase (g/100 g) (b) from PLS models of the NIR spectra of 1170–1290 nm using the samples taken after 24 h of marinating, (-line) best fit through data, green line. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

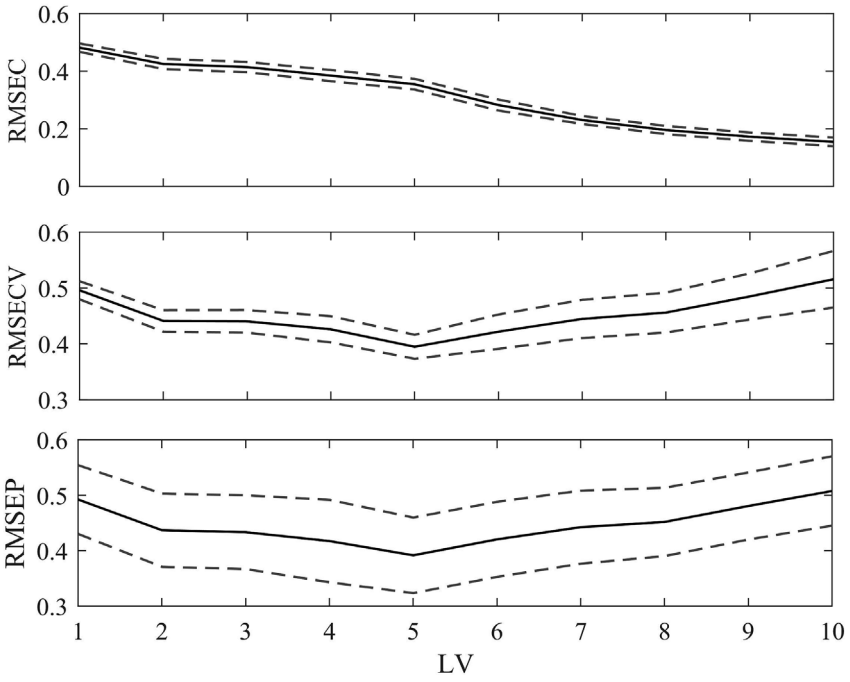

Fig. 8. Predictions errors RMSEC (g/100 g), RMSECV (g/100 g) and RMSEP (g/100 g) vs. the model complexity from external validation of WPS model. Average error (−) and the standard deviation (- - -) from 100 repetitions, LV: latent variables.

Declaration of interest

None.

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